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Optimization of Environmental Conditions to Maximize Carbon Dioxide Sequestration through Algal Growth

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OPTIMIZATION OF ENVIRONMENTAL CONDITIONS TO MAXIMIZE CARBON DIOXIDE SEQUESTRATION THROUGH ALGAL GROWTH

THESIS

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Abstract

The micro-alga *Chlorella vulgaris* was cultivated under a variety of environmental conditions in various culture media solutions to assess and optimize growth rate and biomass productivity. Efforts during this work investigated growth parameters at the micro-scale in an air-lift bubble system with the goal of interpreting performance characteristics that can be applied to a larger serpentine tubular Photo-bioreactor. Maximum growth rates and biomass yields were 0.65 d^{-1} and 2.003 g biomass/L and achieved in seven days using urea in de-ionized water under a 24:0 Photoperiod (Light:Dark). Additionally, growth rates and biomass yields of 0.65 d^{-1} and 1.964 g biomass/L were achieved over the same time period using commercial fertilizers in Charcoal Filtered Tap Water, indicating that the alga is very robust and tolerant of a wide range of environmental conditions, including nutrient composition and water type.

CO_2 tolerance was investigated to determine the utility of the alga in power plant flue gas remediation schemes. The alga grew in all CO_2-in-Air concentrations between ambient air and 50% CO_2 with maximum growth occurring at concentrations between ambient levels and 20% CO_2-in-Air. However, reductions in growth rate and biomass yield were observed at CO_2-in-Air concentrations between 20% and 50%, indicating some level of pH induced toxicity. Greatest growth was observed in the culture grown on 15% CO_2-in-Air, indicating this particular alga may be appropriate for power plant flue gas remediation (13-16% CO_2 in flue gas).

To My Wife and Children

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Kenneth M. Karcher

Table of Contents

Page

Page

Page

List of Figures

List of Tables

Table Page

Table Page

Table Page

OPTIMIZATION OF ENVIRONMENTAL CONDITIONS TO MAXIMIZE CARBON DIOXIDE SEQUESTRATION THROUGH ALGAL GROWTH

I. Introduction

Background

Emissions of carbon dioxide (CO_2) are predicted to increase throughout the century leading to increased concentrations of CO_2 in the atmosphere. Current levels of atmospheric CO_2 rest at ~ 386 ppm (NOAA, 2009). At the current rate of increase, it has been postulated that CO_2 levels could reach 1000 ppm, a point of irreversible climate change (Matthews, 2008). Scientists believe these values to be possible due to the increasing demand for fossil fuels and increasing world-wide populations. While there is still much debate on the actual effects increasing CO_2 levels will have on global climate, populations, and resources, many scientists agree that the projected increases will have a profound effect on the environment. In fact, the United Nations predicts a gain of 0.2-0.4° C per decade through the year 2100 culminating in a 3-5 °C rise in global temperatures (United Nations, 2007). Global temperature increases could cause sea levels to rise, freshwater sources to become scarce, and traditional agricultural regimens to disappear.

Most of the anthropogenic emissions of CO_2 result from the combustion of fossil fuels for energy production. Accordingly, the DOE estimates that consumption of fossil fuels within the U.S. will "increase by 27% over the next 20 years" (Figueroa et al., 2008). Moreover, highlighting the urgency for a global solution, the combined emissions from both China and India will more than triple U.S. emissions by the year 2030. Meeting this increasing demand for energy without increasing CO_2 emissions requires

more than a mere increase in energy production efficiency. The situation requires a comprehensive plan to more efficiently utilize all of the existing sources for energy while sequestering, capturing, and storing the carbon emitted through the global energy system. Carbon Capture and Storage (CCS) could play a major role in reducing atmospheric CO_2 emissions through efficient and responsible fossil fuel usage and recycling.

Most CCS methods center on the power plant, and rightfully so. Fully one-third of U.S. Carbon emissions generate there (DOE, 2009). These point sources are the easiest to target for the reduction of emissions. Currently, there are three generic types of CCS technologies applied to power plants; Post-Combustion, Pre-Combustion, and Oxy-Combustion technologies. Each succeeds to varying degrees but all come at extreme cost. Estimates for removing CO_2 from a conventional coal-fired power plant are extensive; there is expected to be a 5-30% parasitic energy loss, a 35-110% increase in capital cost, and a 30-80% increase in the cost of electricity (DOE, 2009). To truly achieve global accord and initiate global action, a more cost-effective approach must be agreed upon and propagated throughout the world.

One such approach that shows great promise is the use of Algal Biomass; some would suggest that this would complete the fossil fuel cycle, as algae are understood to be the progenitors of our current oil based fossil fuel stocks. As primary producers, the algae can play a vital role in Carbon sequestration, called to action to pull CO_2 from the atmosphere and sequester it in their biomass as they did billions of years ago. The sequestration value can be quantified as follows, based on mass balance; for every pound of algal biomass created, 1.83 pounds of CO_2 are sequestered (Chisti, 2007). After growth, portions of this biomass (mainly the lipids) are harvested and converted into bio-

diesel. Through photosynthesis, algae could sequester CO_2 with 6% solar efficiency in an "open" environment. Higher order plants only sustain a 0.2% solar efficiency (Nakamura et al., 2002). In a "closed" system, where nutrients and light content are strictly monitored, algae could achieve even greater levels of efficiency, up to 10 times the amount seen in an "open" system (Usui and Ikenouchi, 1997).

Problem

As the world leader in coal resources, the United States holds 27% of the world's known coal reserves. Published by the Energy Information Administration (EIA), the Department of Energy (DOE) estimates U.S. known reserves of 489 million short tons; of which, only 40% is mineable. As the U.S. weans itself from its dependence on foreign oil, coal has received growing interest and attention as a resource to produce synthetic gasoline, known in the industry as *syn-gas*. Referred to as the Fischer-Tropsch (F-T) process, using high pressure steam, coal is gasified to create Hydrogen gas (H_2) and Carbon Monoxide (CO), which is then shifted with a catalyst to create gasoline. While the F-T process accomplishes the goal of reducing the country's dependence on foreign oil, it perpetuates the problems encountered with increasing GHG emissions, as this two-phase process yields twice as much CO_2 as does burning coal alone for its energy value. Accordingly, in compliance with Section 526 of the 2007 Energy Independence and Security Act, federal agencies are prohibited from buying / using fuels created through synthetic processes that produce more GHGs than would be produced with traditional fuels. The U.S. Air Force Research Lab (AFRL) is currently researching synthetic fuels production processes and F-T fuels for use in Air Force airplanes and vehicles.

Therefore, it is essential that efficient CO_2 capture technologies are developed for use in tandem with Air Force synthetic fuels production. Combining micro-algae technologies with existing technologies for coal gasification will greatly enhance our country's energy independence while increasing our role in the world as a responsible emitter of CO_2 to the atmosphere.

While extensive research and data exist regarding the optimization of various algal species growth under specified environmental conditions, they tend to focus on a specific end state. For instance, all data tend to point to the use of de-ionized (D.I.) or distilled water for growth conducted at the micro-level while varying one or two environmental conditions to test a hypothesis regarding maximizing growth or lipids productivity. Conversely, the data is relatively sparse regarding the optimization of a cradle-to-cradle cultivation strategy beginning with the water source utilized in the photo-bioreactor (PBR).

Considering the scope and size of most PBRs, specifically the 3800 L PBRs at the University of Dayton, it becomes logistically burdensome and cost prohibitive for the transportation of water to the PBR, utilization of algal nutrients within, and the resulting harvest for bio-fuels and other commercial applications. Therefore, this research will begin by investigating the differences in growth observed in *Chlorella (C.) vulgaris* through the use of different water types. Research will then progress to other environmental conditions in an effort to determine optimal conditions through which to maximize algal biomass and lipid content for future uses in carbon sequestration and in the production of bio-fuels.

Research Objectives

Literature regarding the optimization of growth parameters for algae in large-scale PBRs is minimal. Even if the literature was robust, the fact remains that each PBR is unique and performs as a function of the environment within which it resides. Mass algal cultivation has only recently begun at the University of Dayton. Therefore, it is essential to determine the optimal growth parameters in order to maximize algal biomass production within the PBR.

Research Questions

1. How is the alga affected by the use of different water sources? The PBRs maintained by the University of Dayton can run on tap water. It is essential to determine if the free chlorine or nutrients / contaminants within the tap water plumbed to the University of Dayton has a detrimental affect or a non-effect on mass algal cultivation within the PBRs.
2. How does algal exposure to increasing CO_2 concentrations affect their growth, as compared to those concentrations available in the ambient atmosphere (0.04 % v/v)? An appropriate algae species must be capable of growing under high CO_2 concentrations (~15%), similar to that found in power plant flue gas. Does extreme pH, when driven by high concentrations of CO_2, negatively affect algal productivity?
3. Is there an appreciable difference in growth rate for the alga grown using commercial fertilizers over those media specialized for algal mass culture?

4. Does photoperiod play an important role in algal growth? Is there a benefit to the alga associated with exposure to light for shorter time periods per day versus continuous exposure?
5. How is growth affected during scale-up through the introduction of algae at varying degrees of culture dilution?
6. Do alternative forms of Nitrogen enhance or adversely affect the growth of *C. vulgaris*, as compared to the standard Nitrogen type listed in Bold's recipe?

Research Methodology

This project investigated the durability of the algae and their ability to sequester CO_2 while varying several environmental parameters. The intent was to optimize CO_2 sequestration for a specific algal species through micro-level experiments in a laboratory setting while modifying various environmental conditions (water types, CO_2 concentration, photoperiod, and inorganic nutrient composition).

The primary measure used to determine growth was the culture's optical density. Based on photometric law, each layer in the algal suspension scatters light in a manner that is proportional to the algal dispersion concentration. This method provided a rapid and simple process for the estimation of algal concentration. Due to the presence of photosynthetic pigments, it is important to conduct the measurement outside of the range of wavelengths where these pigments absorb. For this reason, as Becker (1994) suggests, an absorbance wavelength of 550 nm was used. Prior to each stage of the experiment, the absorbance for each species at 550 nm (A550) versus the dry weight at different concentrations was plotted to determine the amount of algal biomass in a particular sample.

Algal growth and productivity was estimated through the use of the exponential growth and decay equations presented as appropriate for algae by Guillard (1973), as long as the results could be linearly correlated with cell mass (as discussed before). By solving the equation for the growth rate and using observed optical densities, rates of growth per day and the doubling time for each culture were estimated. This method took advantage of the underlying principles discussed in the preceding paragraph regarding A550 measurements.

One other method applied during this research was dry-weight estimation. Each time the alga was re-cultured from an agar slant, a calibration curve was created relating biomass concentration to the alga's absorption at 550 nm. In this method, a volume of culture uniformly mixed in suspension was gathered and filtered through micro-pore paper, rinsed with distilled or de-ionized water, dried, and then weighed. This measurement facilitates use of the standard growth curve equation throughout this research to define cell concentration.

Scope of Research

This research determined some of the most important environmental conditions with which to optimize algal growth for a particular green algal species found in Southwestern Ohio. It also determined the species' potential for use as a candidate for CO_2 sequestration and bio-fuels production.

This research could not investigate even a fraction of all algal species. Thus, the particular species reported here should not be considered a primary candidate on a short list of candidates for CO_2 sequestration and bio-fuels production. Additional comparative

research should be conducted with other species to determine *C. vulgaris'* rank among each candidate.

All experiments were performed under controlled laboratory conditions and did not attempt to replicate all of the environmental conditions and variables encountered by the algae in the open environment. Therefore, the results reported here should be viewed as a starting point for outdoor cultivation. However, these results may be appropriate in a closed and controlled system, like the system present at the University of Dayton Research Institute. Additionally, many of the results reflect findings of micro-scale cultivation. The conclusions presented in Section 4 and 5 of this report should be corroborated against large scale, or macro-level algal cultivation as many environmental parameters will affect culture growth in a large PBR that are not a concern at the micro level. These parameters include, but are not limited to, excessive O_2 levels, culture pH, mutual shading of algal cells, and Photo-Inhibition.

II. Literature Review

Overview

This section will review the history of green algae's place in carbon sequestration and its more recent use as part of a world-wide strategy for the production of bio-fuels and the reduction of Greenhouse Gases (GHG), specifically CO_2. It reviews algae's role in the formation of the Earth's atmosphere that we currently enjoy and identifies plausible methods in use today to maintain tolerable levels. It looks at the problems encountered with the algae's mass production and the associated parameters that can be controlled to optimize its growth. This chapter will review the biology of the species investigated, as well as the manner in which photosynthesis is accomplished within its structure. Additionally, the species' participation in current mass culturing schemes, as well as its ability to fix inorganic carbon while manipulating cell contents for the production of bio-fuels, will be reviewed. Finally, this section identifies and discusses algae's role in the bio-fuels strategy, as compared to other bio-fuels production options.

History

Algae have played significant roles in the Earth's development for billions of years. Most notable is their role in the generation of the first oxygen atmosphere. Ancient Cyanobacteria and their descendents are responsible for producing important fossil fuel deposits and the massive carbonate rock formations that led to the reduction of atmospheric CO_2 levels in a process known as photosynthetic sequestration. Modern algae produce about half of the atmosphere's O_2 and powerfully influence the cycling of carbon, nitrogen, phosphorus, sulfur, and other elements, affecting other organisms in

diverse ways. Like most eukaryotic algae and plants, modern cyanobacteria influence the Earth's atmospheric chemistry through the production of O_2 and the reduction of CO_2 in a process called oxygenic photosynthesis. Eigenbrode and Freeman, in their article investigating ^{13}C levels in Archean substrates, discuss the fossil, geochemical, and molecular evidence that indicates the cyanobacteria were the first oxygenic photo-synthesizers at about 2.45 billion years ago (Eigenbrode and Freeman, 2006). They observed dramatically different ^{13}C levels in shallow waters (photic zone) in respect to the deeper substrates. Buick and Brocks et al. conducted similar work in their research tracking the history and timeline of early cyanobacteria and their role in oxygenic photosynthesis. Thus, the evolutionary origin of cyanobacteria and their appearance in time were pivotal events in the history of life on Earth (Graham et al., 2009). Considering the nature of evolution, it would take approximately 1.7 billion years for the Earth's atmosphere to stabilize at levels humans now recognize. These pre-eminent cyanobacteria are believed to have first appeared around 2.7 billion years ago (Buick, 1992; Brocks et al., 1999). At that time, Earth's atmosphere was much richer in CO_2 than it is today, and devoid of O_2. Life processes during these early time periods were mostly characterized by relatively inefficient anaerobic processes that generated the cellular Adenosine Tri-Phosphate (ATP) needed to run the organism. Over the next several hundred million years, O_2 produced by early cyanobacteria accumulated in the atmosphere to levels that afforded several benefits. First, at about 2.4 billion years ago, O_2 was abundant enough that organisms could use it as an electron acceptor in more efficient aerobic respiration (Eigenbrode and Freeman, 2006). This change fostered the evolution of modern Eukaryotes at about 1-2 billion years ago, which is believed to have

initiated the evolutionary processes through which multi-cellular animals, fungi, and plants later arose, a process called endosymbiosis and is discussed in the following paragraph. Subsequently, as these aerobic processes became more and more pronounced, atmospheric O_2 began to interact with incoming Ultra-Violet (UV) radiation. These chemical interactions generated a stratospheric ozone (O_3) shield at about 1 billion years ago that would prove to be sufficient protection for some surface life to exist without sustaining photo-induced cellular damage. Thus, eukaryotic life could exist in the surface waters of the Earth and on land, conditions that exponentially increased CO_2 fixation and O_2 production.

An advancement in algal evolution, Eukaryotes enjoy higher metabolic rates than their predecessors. Through phagotrophy, early Eukaryotes consumed cyanobacteria to sustain their life processes. As the Eukaryotes became more abundant, through a process known as endosymbiosis, the early Eukaryotes began to assimilate the cyanobacterial processes within their own structures, leading to a heterotrophic lifestyle. Instead of completely breaking down the cyanobacterial cellular components for one time ingestion, the cyanobacteria were retained within a Eukaryotic food vacuole for a continuous stream of organic carbon. Over time, the cyanobacterial cell components evolved into the plastids we observe today in the algae. All of the plastids present in modern day protists and plants arose through early Eukaryotic primary, secondary, and tertiary endosymbiosis (Kim and Archibald, 2008). Together with the cyanobacteria, early eukaryotic algae continued to produce O_2, with the result that atmospheric levels had nearly reached modern levels (21%) by 550 million years ago. The resulting changes to what is now

considered an O_2 rich atmosphere are responsible for the rise and maintenance of diverse communities of multi-cellular marine communities and land-based plants.

Microalgae

Overview

Since the dawn of our modern world (atmosphere conducive to multi-cellular organism growth), the microalgae have played an essential role as primary producers. Today they continue to play important roles spanning multiple disciplinary fields. Algae are cultured for use as food supplements, aquaculture feed, agricultural feed stock, fertilizers, waste treatment systems, and for bio-fuels. Like any other photosynthetic entity, microalgae utilize the energy of the sun to increase their growth. Biomass is produced according to the following reversible reaction:

$$6\ CO_2 + 6\ H_2O + h\nu \rightarrow C_6H_{12}O_6 + 6\ O_2$$

Due to shortages of fossil fuels and the recent interest in Greenhouse Gas (GHG) emissions, this process used by the microalgae is being investigated with greater frequency for its role in several remediation processes and for bio-fuels production (Hill et al., 2006). Of the alternatives, bio-diesel is the most promising.

Current sources of bio-diesel include soybean oil, rapeseed oil, palm oil, corn oil, jatropha, animal fats, and waste cooking oil. However, considering the scope of the world's energy uses, these sources cannot possibly replace the fossil fuels currently in use. Some research has been done in this area. Considering the average oil yield per hectare from the various crops, Chisti reported land area values required to satisfy America's biodiesel needs, which are reported as 0.53 billion m^3 per year (Chisti, 2007).

These values are listed in the table below:

Crop	Oil yield (L/ha)	*Land Area Needed (M ha)	*Percent of existing US cropping area
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil Palm	5950	45	24

* For meeting 50% of transport fuel requirements in the United States

*adapted from Chisti, 2007

Table 2.1: Comparison of Bio-Diesel Sources.

For instance, from the table it is suggested that to meet even 50% of America's fuel transport needs, 846% of existing cropping area would be required if cultivating corn-based fuels. Likewise, the other crops listed above require substantial cropping area for 50% of fuel transport alone. Clearly, as practiced now, oil crop cultivation cannot significantly contribute to the replacement of petroleum derived liquid fuels. However, the outlook changes considerably when microalgae are considered. From the table below and considering algal productivity, a mere 1-3% of existing United States cropping area would be required to replace 50% of America's fuel transport needs (Chisti, 2007).

Crop	Oil yield (L/ha)	*Land Area Needed (M ha)	*Percent of existing US cropping area
Microalgae(a)	136,900	2	1.1
Microalgae(b)	58,700	4.5	2.5

* For meeting 50% of transport fuel requirements in the United States

(a) 70% oil (by wt) in biomass

(b) 30% oil (by wt) in biomass

*adapted from Chisti, 2007

Table 2.2: Comparison of Algal-Based Bio-Diesel.

Oil yields above for microalgae are based on experimental procedures using photo-bioreactors (PBR). If the results observed by Chisti and others can be replicated, microalgae could be the only viable source of bio-diesel with the potential to replace fossil fuels as the primary fuel used for transportation.

There are several factors contributing to the attractive oil yields put forth by microalgae. First, the algae grow very rapidly, sometimes doubling their biomass every 4-6 hours. Second, their oil contents can approach 80%, depending on the species of algae and the nutrient conditions observed in the culture (Metting, 1996; Spolaore et al., 2006). Third, microalgae do not compete with traditional food crops, as corn-based ethanol does.

Additionally, when grown in outdoor cultures, the algae can be grown in tandem with wastewater treatment systems using the waste water stream effluent as a water and nutrient source. Also, the algae can be designed to operate in the downstream processes of a coal-fired power plant, designed to utilize the flue gas emissions for growth (Sawayama et al., 1995; Yun et al., 1997). The benefits are two-fold, reduction in GHG emissions from the power plant stack, and an increase in algal biomass for bio-fuels production. Of course, as photosynthetic organisms, algae require sunlight for growth. Many areas of the U.S. currently considered untenable because of their lack of water, resources, and infrastructural support can be used to cultivate the algae. The desert Southwest is becoming increasingly attractive due to the large amounts of sunlight received over the course of a year. Additionally, many of these areas have never been considered an option for cropland. Thus, their use in algal cultivation would not interrupt existing cropland used for food production.

Photosynthesis and Irradiance

As mentioned above, algal biomass is created by autotrophic and heterotrophic algae through photosynthetic processes. Whether in its natural or artificial form, irradiance induced photosynthesis is absolutely critical to any algal cultivation strategy. As Bryant and Frigaard explained, light emitted by the sun (or some other irradiance source) is captured by the antennae of the phototrophic algae via resonance energy transfer to the Photosystem I and II reaction centers (Bryant and Frigaard, 2006). Free energy is transferred through a series of electron events culminating in the conversion of the original light into the energy of Nicotinamide Adenine Dinucleotide Phosphate (NADPH). Calvin describes this process as leading to a proton gradient causing the formation of ATP in an amount that matches the requirements for conversion of ATP, NADPH, and CO_2 into phosphorylated carbohydrates (biomass) (Calvin, 1989).

It has been reported that the Earth receives approximately 1.2×10^{17} Joules/second (watts) of sunlight m^{-2} d^{-1} (Ramaswamy et al, 2001). Of this solar radiation, only those wavelengths between 400 and 700 nm are available for photosynthesis, known as Photosynthetically Available Radiation (PAR). These wavelengths correspond to about 45% of total radiation. Internal plant processes reduce this efficiency again to around 11% and, due to factors such as availability of sunlight, water, and nutrients, the overall photosynthetic efficiencies reach only 0.1 – 2% for terrestrial crops and 3-6% for algae. Higher efficiencies for algae result from their greater surface area (submerged in water) in contact with vital nutrients. Even so, the reported efficiencies have been sufficient for billions of years to create the habitable planet we currently enjoy. Productivity of the microalgae culture is thus determined in

large part by both the light input and the efficiency through which light is utilized in Photosystem I and II to convert CO_2 into biomass.

Like other living organisms, algae are affected by the intensities of light to which they are exposed. Exposure to too little light may prohibit logarithmic growth, resulting in decreased biomass productivity. Exposure to too much light inhibits growth and kills the organism. This is known as photo-inhibition and is generally a reversible effect, if recognized early. Photo-inhibition results when maximum growth rate is achieved given an unchanging suite of nutrient conditions, such as would exist in a PBR or pond. Specifically, photo-inhibition is defined as no further growth in an algal culture as a result of increasing light intensities. Up to the light saturation value (increasing irradiance permits increasing growth), algae will grow exponentially with increasing irradiance. Above this light saturation value, a further increase in irradiance actually reduces the biomass growth rate. Most algae, including the ones investigated in this experiment, become photo-inhibited at irradiance levels slightly greater than the light level at which their growth rate peaks. It is therefore important to determine the light saturation value for the algae in order to avoid photo-inhibition and to maintain algal cultures below this level. Theoretically, this would allow for continuous growth of the algae. Chisti emphasizes the importance of avoiding excessive light intensities while culturing algae in his 2007 article "Biodiesel from Microalgae." Many others have investigated the light inhibiting effects on various algae species. Most notably were Constantine Sorokin and Robert Krauss. Their pioneering work defined irradiance conditions for maximizing productivity in various algae species. They conducted experiments to determine the light saturation and photo-inhibition levels for five different

algal types. Growing these cultures at various light intensities over a time period and then plotting each species' growth rates against the respective irradiance level, they were able to determine the light saturation and photo-inhibiting intensity for each species. For *C. vulgaris*, they concluded that increasing the light intensity above 36-44 μmol m^{-2} s^{-1} did not result in any increase in growth rate. In fact, growth rate steadily declined through intensities of 290 μmol m^{-2} s^{-1} at which point an increase in intensity marked a steep decline (Sorokin and Krauss, 1958). Sorokin and Krauss continue in their article to describe the use of half-saturation values in order to maximize productivity. Using an irradiance level of between 18-20 μmol m^{-2} s^{-1} ensures that (given no other limiting conditions) the algal culture will remain in exponential growth, yielding maximum biomass.

Carbon Sequestration

As a means of reducing CO_2 levels in the atmosphere, various carbon sequestration methods have been investigated. Considering that over one-third of world-wide CO_2 emissions originate from coal-fired power plants (Herzog, 2001), many of the sequestration strategies have focused on expensive carbon capture technologies and transportation of the power plant flue gas to long-term sequestration sites, such as formerly mined areas, saline formations, and deep ocean areas. However, these processes are very expensive, up to 2¢ kWh^{-1} for capture alone (Herzog, 2001). Associated costs are much higher as distance to the sequestration site increases from the power plant. Caleb Stewart and Mir-Akbar Hessami conducted an exhaustive review of the various methods for carbon capture and storage (CCS) in which they detail the benefits and detriments of each capture and storage option (Stewart and Hessami, 2004). Of the

options, algal sequestration is the most viable because of the array of products obtained from the process; these products include bio-fuels, H_2 production, health food / aquaculture feed, bio-molecules, fertilizers, and industrial materials (Skjanes et al., 2007).

As a required component for biomass production in algae, it is suggested in the literature that algae should be a part of the global strategy for atmospheric reduction of CO_2 and for bio-fuels production. Accomplishing both objectives simultaneously would be ideal. Using the CO_2 from fossil fuel-fired power plants as the primary feedstock for algae will provide a large sink for previously pre-destined CO_2 and present considerable cost-savings to the power producer and power consumer. In accordance with the end-state presented in the University of Dayton Research Institute (UDRI) Statement of Work (SoW), it is important to test the algae's robustness for high levels of CO_2. The goal of the program being to utilize the PBRs at each of Wright-Patterson Air Force Base's coal-fired power plants or at the Fischer-Tropsch plant (to be built in the future), algae growth should be measured against varying concentrations of CO_2 in order to simulate values up to 15%, or that which is typical in coal-fired power plant flue gas. Additionally, given flue gas temperatures, it is beneficial to identify an optimal temperature range for growth of a particular species of algae. These results are essential in determining the appropriate species for use in the CCS and Bio-Fuels production strategy at Wright-Patterson Air Force Base, as they indicate the ability of the particular species to assimilate CO_2 from flue gas. Several researchers have studied various *Chlorella* species and reported their CO_2 tolerance levels as appropriate for average flue gas concentrations (Hanagata et al., 1992; Maeda et al., 1995; and Zeiler et al., 1995). Additionally, research has been conducted on various *Chlorella* species regarding their tolerance for high temperatures.

Hanagata et al. investigated temperature tolerance in *Chlorella spirulina* and found that the organism could tolerate high CO_2 levels but not temperatures above 45°C (Hanagata et al., 1992). However, flue gas temperatures can reach 120°C. To date, only blue-green algae species have demonstrated growth under high temperature regimes up to 120°C; specifically, researchers have investigated the species *Cyanidium caldarium* and determined it suitable for temperatures up to 100°C (Seckbach et al, 1971). Therefore, to utilize a *Chlorella* species for flue gas sequestration, the flue gas would first have to be cooled from 120°C to below those levels observed by Haganata et al.

Considered separately as an end-state in itself, as opposed to a means through which bio-fuels are produced, algae are quite capable of sequestering large amounts of CO_2. In fact, just as some species are used to optimize bio-diesel production, some species are grown to optimize CO_2 sequestration. With a generic biomass formula of $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$, approximately half of the dry weight of algal biomass is carbon (Grobbelaar, 2004). In an autotrophic culture, all carbon is derived from CO_2. Therefore, producing 100 tons of biomass fixes approximately 183 tons of CO_2 (Chisti, 2007). The Department of Energy annual report for the year 2000 put the per person value of CO_2 power sector emissions in the U.S. at 9 tons per person per year. Total output for the U.S. was estimated at 2.245 million metric tons (DOE, 2000). Cheng et al. have observed fixation rates in the laboratory (10 L PBR) using *C. vulgaris*, that, when transposed over a larger 100,000 L PBR, and assuming production rates could be maintained as in the 10 L bench scale project, biomass production values of 114 tons CO_2 captured in biomass per year were tabulated (Cheng et al, 2006). Cheng et al. achieved these values using environmental conditions not unlike those used in these experiments;

1% CO_2 in air mixture, 25-30°C, and irradiance of ~150 μmol/m^2 s. Given theoretical values as such, and considering the *% lipid content* values listed in the opening paragraphs of this section, it is easy to imagine the benefits of combining algal technologies with power plant flue emissions in a strategy for CO_2 sequestration.

Chlorella vulgaris

The unicellular photosynthetic microalga *C. vulgaris* is a member of the Class *Trebouxiophycea* of the Phylum *Chlorophyta*. It is spherical in shape, and ranges from 2 – 10 μm in diameter. A green alga, it contains the green photosynthetic pigments chlorophyll-a and chlorophyll-b within its chloroplast. While capable of autotrophic growth, it is routinely cultured with a small amount of nutrients. In fact, some researchers have grown *C. vulgaris* heterotrophically and have achieved interesting results. Typical growth observed can reach as high as 0.99 day^{-1} and achieve between four and six doublings per day, given sufficient nutrient conditions.

Interest in *C. vulgaris* began in the early 1950s when it was recognized first by the Japanese as an adequate protein source. Later, investigation by the U.S. regarding its use as a food supplement for the space program and for alternative fuels during the oil crisis of the 1970s was initiated. However, the first large-scale production began in the 1960s in Japan. By 1980, after the U.S. had largely forgotten about mass production (mostly because the oil crisis of the mid-1970s had ended), there were 46 large scale factories in Asia (mostly Japan) producing more than 1000 kg of algae (mainly *Chlorella*) species per month (Spolaore et al., 2005).

C. vulgaris has a nutrient composition of 51-58% protein, 12-17% carbohydrate, and 14-22% lipid. From a protein and lipid perspective, these values compare favorably to those other traditional sources for milk and soy (Spolaore et al, 2005). Because of these values, and because they can be manipulated to maximize certain components, *C. vulgaris* is a widely used nutritional supplement.

Additionally, research has been conducted regarding the utility of *C. vulgaris* as a bio-fuels option. Specifically, full fatty acid profiles have been published regarding its use as a bio-fuel substitute (Gouveia and Oliveira, 2008). While there are better options for bio-fuels substitutes (*Botryococcus braunii*, *Neochloris oleabundans*, etc…), *C. vulgaris* presents itself as an algal species that is not only robust in its tolerance of various environmental factors, but as already mentioned, has utility across many industries. When its oil is blended with other algal species' oil or diesel fuel itself, it presents an adequate bio-fuel substitute. It is for these aforementioned reasons and because of its ubiquitous nature that *C. vulgaris* was investigated in this study.

Lipids Production

As discussed before, the chemical composition of the algae is not a constant factor but varies over a range of nutrient conditions. Several factors influence the proportion of chemical constituents within the algal biomass. Most notable among the environmental factors are light and dark cycles and the nutrients carbon and nitrogen. When the goal of biomass cultivation is oil production, researchers must maximize lipid content within the algal cell. To maximize lipids production, one must effectively stress the algae. Stressing the algae retards algal reproduction rates and focuses cell energy toward life sustaining processes within the cell. Considering these reasons, Becker suggests

cultivating algae for bio-fuels production in two stages; first, algae are grown under normal conditions to first maximize biomass growth rate, and then second, nitrogen is removed or the algae are otherwise stressed in an effort to force the algae species to convert carbohydrates into lipids (Becker, 1994).

Many microalgae grown (stressed) under nitrogen limiting conditions show increased lipids production within their cells. For instance, Converti et al. cultured *C. vulgaris* under normal conditions, and then deprived the culture of nitrogen (as $NaNO_3$). They observed a tripling of lipid content without any change in algal growth rate (Converti et al., 2009). Additionally, it appears to be clear across the literature that lipids are maximized through nitrogen deprivation. However, the results reported by Converti et al. regarding growth rate seem to be the exception vice the rule. Most observers see reduced growth rates coupled with nitrogen deprivation. In fact, Illman et al. observed lower growth rates with increased lipid content from 18 to 40% under nitrogen limiting conditions (Illman et al., 2000).

Heterotrophic growth of algae has been shown to yield higher lipid contents in several algal species. This topic has not been observed in great detail; in fact, biodiesel production from heterotrophic algae had never before been investigated until Miao and Wu published their research in 2006. Using *C. protothecoides*, Miao and Wu (and later Xu, Miao, and Wu) were able to demonstrate that the algae will produce large amounts of lipids as percent of dry weight when glucose is added to the culture medium. They observed lipid content of 55% heterotrophically vice 14.5% autotrophically, without altering the nitrogen content (Miao and Wu, 2006; Xu et al., 2006). Apparently, the algae continue to metabolize carbon (from the glucose) when light is removed from the culture,

creating biomass instead of losing biomass through respiration. These results are groundbreaking as it appears lipid content for any species may be maximized without sacrificing growth rate, as one usually sees when depriving a culture of nitrogen.

Growth Kinetics and Measurement Methods

Growth

Growth can be identified as any form of biomass accumulation in the algal culture. Typically, for unicellular algae, growth is estimated from the culture with an understanding that the growth parameter being followed increases as a fixed percentage of the total unit time. When the parameter of interest is cell number or a proxy measure (fluorescence, biomass dry weight, and optical density) that is directly proportional to cell number, these methods provide an estimate of the population growth rate when they can be shown to be linearly correlated with cell number or biomass (Wood et al., 2005).

However, linear correlation is only satisfied when the algal culture exists in its balanced or exponential growth phase. For every culture, there is a period of acclimation that exists for the species where growth rate is quite variable. In a closed system, where food is limited, all algae progress through several different phases:

Phase	Description
1	Adaptation/Lag Phase
2	Accelerating Growth Phase
3	Exponential/Balanced Growth Phase
4	Decreasing Log/Linear Growth Phase
5	Stationary Phase
6	Accelerating Death Phase
7	Log Death Phase

Table 2.3: Phases of Growth for Homogenous Algal Batch Culture.

When inoculated in new medium, algae must first adapt to their new surroundings. During this time, growth progresses slowly. The algae progress through several generations, perhaps up to 20, as they adjust to their environment (new medium rich in nutrients, temperature, light, and water type) (Wood et al., 2005). Upon entering Phase 2 and 3, the algae have adapted to their surroundings, light and nutrients are no longer limiting, and the algae progress into exponential growth. Phase 3 is the most interesting to researchers as this is the phase where growth rate is calculated. The increment in algal biomass per time is proportional to the biomass in the population at any given point in time according to the equation:

$$dn/dt = rN \quad \text{(Eq. 2.1)}$$

the solution to which is:

$$N_t = N_0 e^{rt} \quad \text{(Eq. 2.2)}$$

where r is the exponential growth rate of the population, N_t is the population at time t, and N_0 is the initial population. As Becker describes, during this phase a steady-state continuum is observed and the plot of the logarithm of cell mass (or other proxy measure) yields a linear increase with time. During Phase 4, growth has occurred to such an extent that mutual shading of cells occurs and nutrients become limited. This effect reduces the growth rate and the increase in algal biomass becomes linear. Phase 4 concludes when respiration outweighs photosynthesis, nutrients become deficient, or toxic waste buildup in the sample becomes significant. Phase 5 is characterized as the stationary phase of growth, or the maximum attainable concentration of algal biomass in the specified closed system. Without adjusting nutrient levels or sub-culturing the algal suspension, the culture will proceed to Phase 6. Phases 6 and 7 mark increasing cell death and

disappearance of cells. Depending on the location of the culture within these phases, recovery of the algal suspension may be irreversible.

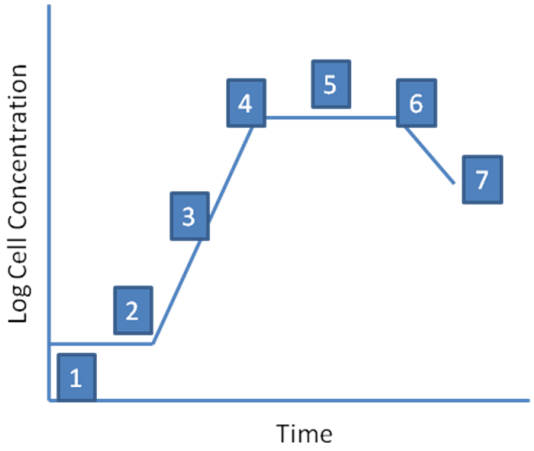


Figure 2.1: Growth Phase Diagram.

Measurement Methods

To determine growth rates of the algal suspension, calculations are made while the culture is in the exponential growth phase. Turbidity and Dry Weight estimations are two of the more prominent methods used by researchers. Both methods were used in this research. For determining cell growth rates, Becker suggests creating a *standard curve* correlating absorption of the suspension versus the dry weight at different concentrations. As Becker showed, and as many others have demonstrated (Xu et al., 2006; Liu et al, 2008; Converti et al., 2009) biomass concentration can be related to suspension absorbance. In fact, the amount of light that passes through the suspension will be inversely proportional to the concentration of organisms, in accordance with Beer's Law relating absorption to concentration. Considering the wavelengths where Chlorophyll-a and -b absorb, an Absorbance at 550 nm is recommended and will be used to construct the *standard curve* (Becker, 1994). Use of the *standard curve* yields a linear equation

that compares Absorbance of the suspension at 550 nm to the cell concentration at that particular time. The equation, in its generic form, will appear as follows:

$$y = Ax \quad \text{(Eq. 2.3)}$$

where *A* is the linear regression line fit variable, *x* is the A550 reading and *y* is the cell concentration of the suspension at the particular absorbance *x*.

Dry weight estimation is one of the more common and easier methods to use for the determination of algal growth. Aliquots of algal suspension are measured over different time intervals. This method provides an estimate for the productivity of the culture in suspension and is usually used when determining volumes of CO_2 sequestered or in determining the amount of lipids produced per unit of biomass.

Batch Culture Techniques

When bio-fuels production or maximum carbon sequestration is the goal, maintenance of the algal suspension in the exponential growth phase is critical. There are multiple ways to accomplish this task; however, they all involve dilution of the suspension and replacement with fresh medium. The method has been practiced since culturing began and is generally accepted as the standard method. It involves culturing the suspension into the exponential growth phase, then removing a portion of the culture and replacing with fresh medium. Wood et al. describe this simple process, the goal of which is to ensure the medium remains fresh and the algae in the culture never have to compete with each other for resources. This allows for continuous exponential growth and harvesting of cells.

Summary

Algae have been critical to life's existence on Earth in the past and will continue to be so as we move into an uncertain future with elevated atmospheric CO_2 levels and shortages of life-sustaining fossil fuels. Harnessing these algal processes, manufacturers have constructed ways to utilize biomass to replace a portion of those life-sustaining fossil fuels. However, considering the land area required to continue in that regimen, these processes are unsustainable as they require large amounts of land and compete with food crops. Algae research has exponentially increased over the past 20 years as researchers have focused on natural photosynthetic processes to accomplish two objectives at once, CO_2 sequestration / removal from the atmosphere, and bio-fuels production. It has been demonstrated that algae are capable of rapid growth and significant bio-fuels production. There are many examples presenting various algal species that grow very well under a variety of conditions, that demonstrate a unique robustness under those conditions, and that can accumulate significant biomass through sequestration of carbon. Algal production and cultivation appears to be the only strategy that can alleviate the world's dependence on fossil fuels while not appreciably contributing excess CO_2 to the atmosphere.

III. Methodology

Experimental Design

Microalga and medium.

Chlorella vulgaris (#152075) (hereafter referred to as *C. vulgaris*) was provided by the Culture Collection of Algae at Carolina Biological Supply Company (Burlington, NC, USA). *C. vulgaris* is a eukaryotic photosynthetic organism and, as such, grows rapidly due to its simple structure. Because of their small size and growth habit, they can be considered as members of the phytoplankton community. The culture medium and method were as described by Bischoff and Bold using Bold's Basal Medium (BBM) (Bold 1949, Bischoff and Bold. 1963) supplemented with 4 x $NaNO_3$ concentration. BBM was autoclaved in 250 mL flasks at 121° C for 30 minutes and then placed under a sterile laminar flow hood. The algae were inoculated from agar slants under a laminar flow hood using sterile cotton swabs into 1L Erlenmeyer flasks containing 500 mL BBM. Flasks were fitted with foam stoppers for air exchange and covered with aluminum foil. Cultures were grown autotrophically on atmospheric air at temperatures of 25 ± 1° C with continuous illumination at intensities of 40 µmol m^{-2} s^{-1}, verified by a LI-COR Light Meter (model # LI-250A, serial # LM2-2084). Stock cultures were maintained throughout the experimental time period. This procedure was repeated when necessary to ensure pure culture was maintained as stock.

Exponential Growth.

To ensure that each culture was in the exponential growth phase (log phase) before proceeding with the experiments, growth curves were prepared for each pure

culture (Perkin-Elmer spectrophotometer, Model # Lambda-3B, S/N - 69430) by measuring the absorbance at 550 nm (A550) daily. A550 was plotted against time and growth rates were obtained as Guillard described (Guillard, 1973):

$$dn/dt = rN \quad \text{(Eq 3.1)}$$

the solution to which is:

$$N_t = N_0 e^{rt} \quad \text{(Eq 3.2)}$$

where N_0 is the population size or cell density at the beginning of the time interval being estimated. N_t is the population size or cell density at the end of the time interval being estimated. r is the proportional rate of change vs. time (growth rate) and t is the time of the interval. A continuous plot of these values allowed for easy determination of the exponential growth phase, due to the straight line relationship observed over the time interval.

Determination of Growth.

For each pure culture, a regression equation was prepared, as Becker discussed, during the culture's exponential growth phase. The dry weight of algal cells was measured by filtering an aliquot of culture suspension on pre-weighed 0.2 μm Whatman GF/C filters. The filters were rinsed with de-ionized (D.I.) water, dried for 16 hours at 85 °C, and re-weighed (Mettler H_2O, S/N – 370165, Error – 0.01 mg). A550 measurements were obtained in triplicate and determined by Spectrophotometer, as indicated above. The absorbance was compared with each suspension's respective dry weight. There was a direct correlation between absorbance and dry weight for each pure culture examined and each was expressed by a function:

$y = 0.2057x$ $R^2 = 0.983$ (Eq. 3.3, for Experiments 1-3)
$y = 0.1594x$ $R^2 = 0.995$ (Eq. 3.4, for Experiments 4-6)
$y = 0.1613x$ $R^2 = 0.995$ (Eq. 3.5, for Experiments 7-9)

where *x* is the algal suspension absorbance at 550nm and *y* is the cell concentration (g/L).

Experimental Procedures

Culture System.

Growth experiments were conducted at a constant temperature of 25 ± 1°C, in 250 mL Erlenmeyer flasks fitted with two-holed rubber stoppers. Rubber stoppers were fitted with plastic tubing to allow for CO_2 and O_2 gas exchange. Each Erlenmeyer flask was filled with 100 mL of medium and sterilized in an autoclave at 121 °C for 30 minutes in order to prevent any contamination during the early stages of growth. Aliquots of algal suspension were withdrawn from stock solution, centrifuged at 730g for 7 minutes (Dynac, S/N-103094), taken up in 1 mL of D.I. water, and added to each Erlenmeyer flask. To keep experimental conditions the same, an initial A550 of ~ 0.200 was targeted and achieved for each experimental run.

Continuous light was provided to the cultures by a battery of Cool White and Grow Lux Fluorescent lights under irradiance conditions of 40 µmol m^{-2} s^{-1} (Experiment 6, where various photoperiods were examined, was the exception). A CO_2-in-air mixture was provided to the cultures according to the requirements of each experiment, but typically at 4% (v/v) CO_2-in-air. Compressed air was filtered and then passed through a sterilized D.I. water bath for humidification (to ensure the compressed air did not evaporate or reduce the culture volume) and then mixed with CO_2 in line. CO_2 concentrations were verified daily to ensure that the appropriate percentage of CO_2-in-air

was being provided to the culture. Unless otherwise stated, air and CO_2 flow rates were verified through the use of a Restek 6000 flow meter (S/N-983532).

Light Type	Cool White / Grow Lux Fluorescent
Irradiance	40 µmol m^{-2} s^{-1}
Photoperiod	24:0 (Light:Dark)
CO_2-in-Air (v/v)	4%
Temperature	25 ± 1°C
Flask Volume	250 mL Erlenmeyer Flask (100 mL Medium)
Number of Flasks	3 Flasks per Culture Condition
Medium Type	Bold's Basal Medium w/ 4 x $NaNO_3$
Water Source	D.I. Water (unless otherwise indicated)
Initial Absorbance	~ 0.200 (at 550 nm)

Table 3.1: Standard Experimental Conditions.

Each autotrophic batch cultivation was carried out using n=3 flasks per culture until the algal cultures passed through their exponential stages of growth into their stationary phase. The duration of cultivation was unique to each experiment conducted, and also dependent upon the amount of CO_2 provided to the culture. The CO_2-in-air mixture provided to the culture can best be described as an airlift system where bubbles are introduced through plastic tubing at the bottom of the culture flask. The agitation provided by the bubbles to the culture ensures continuous mixing that under normal conditions prevents settling of the culture suspension to the flask bottom and cell adherence to the flask walls.

The microalgal suspension absorbance was measured daily. All absorbance measurements were conducted in triplicate and related to the culture cell concentration using the appropriate regression equation as described in the paragraphs above. After completion of each growth experiment, the biomass was separated from the medium by centrifugation at 730g for 20 minutes, taken up in fresh medium and set aside for later use or analysis.

Analytical Methods.

In addition to cell concentration, other parameters were investigated and measured for each experiment. Specifically, the amount of CO_2 provided to the cultures was compared to the amount of CO_2 sequestered in the algal biomass. This provided a snapshot of culture sequestration efficiency and is directly related to the algal growth under the conditions prescribed for each experiment. As described in Section 2 of this document, the algae sequester CO_2 at a ratio of 1.83:1 (CO_2:Biomass). Some have advocated that number be as high as 4:1, due to photosynthetic and diffusion inefficiencies. Using the flow rate of CO_2 to the culture and the final biomass concentration, one can easily determine the theoretical values of CO_2 provided to and taken up by the culture. A detailed description of how these calculations were made and referenced is located in Appendix A and is briefly discussed in Section 4. Solving Equation 3.2 for growth rate (*r*), yields the equation:

$$r = \ln (N_t/N_0)/\Delta t \quad \text{(Eq. 3.6)}$$

which yields specific growth rates for each culture while in exponential growth. Determining the doubling time of the culture in exponential growth was calculated using Eq. 3.6 according to the following equation:

$$\text{Doublings/Day} = r/0.6931 \quad \text{(Eq. 3.7)}$$

or through the use of the "Doubling Time" formula:

$$T_2 = 0.6931/r \quad \text{(Eq. 3.8)}$$

Conduct of the Research.

Experiment 1. Growth optimization while varying Water Source.

Considering the large volumes of water required to culture algae in a 3800 L photo-bioreactor (PBR), it was important to determine an appropriate water source for use in the PBR. The University of Dayton's (UD) PBR resides in a location that presents unique logistical problems with respect to water source. Ideally, D.I. would be available in a continuous flow to the PBR. However, presently it is not. Tap Water though, is readily available at the PBR location. Therefore, the suitability of tap water for use in the PBR was investigated. To determine the appropriate water source, *C. vulgaris* was cultured post-inoculation in four different water sources (according to Table B.1); City of Dayton tap water (CDTW), CDTW filtered through a charcoal filter (Whirlpool "Whole House Filtration System", S/N – W10187984), CDTW filtered through a charcoal filter and autoclaved at 121° C for 30 minutes, and D.I. water (control).

Erlenmeyer flasks (n=3) were prepared for each water source (n=4) bringing a total population of N=12 flasks. Irradiance and photoperiod are as prescribed in Table 3.1. Air was provided to the culture at a rate of 500 mL/min while CO_2 was added in-line at 20 mL/min (4%). Stoppers were removed and agitation was halted once per day to measure A550. Cultures were labeled according to the nomenclature depicted below:

Flask #	Experiment 1
A1-3	D.I. Water
B1-3	City of Dayton TW w/ Charcoal Filter
C1-3	City of Dayton TW w/ Charcoal Filter & Autoclave
D1-3	City of Dayton TW

Experiment 2. Algal growth optimization with alternate nutrient type.

Using the Charcoal Filtered Tap Water as the water source for the medium (except the D.I. control), Erlenmeyer flasks (n=3) were prepared for each trial (n=6) creating a total population of N=18 flasks. Experimental Conditions are as prescribed in Table 3.1, with the exception that the culture medium was prepared with water as the base according to Table B.2. Air was provided to the culture at a rate of 800 mL/min while CO_2 was added in-line at 32 mL/min (4%). Stoppers were removed and agitation was halted once per day to measure A550. One trio of flasks was investigated using autoclaved charcoal filtered tap water, to determine the effect that divalent cations (hardness) may have on algal growth. Culture nomenclature is depicted below:

Flask #	Experiment 2
A1-3	D.I. Water
B1-3	City of Dayton TW w/ Charcoal Filter
C1-3	City of Dayton TW w/ Charcoal Filter & Autoclave
D1-3	City of Dayton TW w/ Charcoal Filter & 1 g/L Cal-Mag
E1-3	City of Dayton TW w/ Charcoal Filter & 2 g/L Cal-Mag
F1-3	City of Dayton TW w/ Charcoal Filter & 5 g/L Cal-Mag

Experiment 3. Algae Scale Up Evaluation.

To determine Algal Suspension concentrations that permit appropriate scale-up from micro-level experiments to the 3800 L PBR, Erlenmeyer flasks (n=3) were prepared for each trial dilution (n=4), as listed in Appendix B, Table B.3, creating a total population of N=12 flasks. Standard experimental conditions prevail as per Table 3.1. Air was provided to the culture at a rate of 1600 mL/min while CO_2 was added in-line at 65 mL/min (4%). Stoppers were removed and agitation was halted once per day to measure A550. To ensure that all conditions remained the same during this experiment,

each dilution utilized the same water source as the control (D.I. water). The results of this experiment served to reveal an appropriate culture dilution for direct scale up to the 3800 L PBR that supports and does not inhibit the algae culture's exponential growth. For example, if growth rates of a culture with an initial A550 of 0.025 mirror those with an A550 of 0.200, then direct addition of the more dilute algal culture to a larger scale PBR is possible without adversely affecting the culture, and thus saves time and resources in scaling up the algae culture. Culture nomenclature is depicted below:

Flask #	Initial A550
A1-3	0.200
B1-3	0.100
C1-3	0.050
D1-3	0.025

Experiment 4 & 5. Growth optimization with alternate nitrogen sources as nitrogen.

To examine the algae's affinity for one source of nitrogen over another, Erlenmeyer flasks (n=3) were prepared for different nitrogen sources (n=6) (concentrations are listed in Appendix B, Table B.4) creating a total population of N=18 flasks. The nitrogen sources investigated were $NaNO_3$, $[NH_4]_2SO_4$, NH_4NO_3, Urea $[(NH_2)_2CO]$, and KNO_3. Cultures A, B, C, and D (exception is Experiment 5, Culture D used Prilled Urea as Nitrogen source) were prepared with American Chemical Society (ACS) grade reagents. Cultures E and F are described in subsequent paragraphs. Experimental conditions prevail as per Table 3.1, with the exception of medium type. Refer to Table B.4 in Appendix B for a list of each medium. Air was provided to the culture at a rate of 5000 mL/min while CO_2 was added in-line at 200 mL/min (4%). Stoppers were removed and agitation was halted once per day to measure A550.

During Experiment 4, cultures were allowed to grow without adjustment to a neutral pH in order to determine two things; one, how is pH affected by the use of a particular type of Nitrogen, and two, how does the algae grow in a non-optimal pH environment. During Experiment 5, culture pH was monitored and adjusted each day to neutral pH (~ 6.6) in order to gauge the utility of each nitrogen type as a nitrogen source for the *C. vulgaris*.

Nitrogen levels for each trial were made equal to the control flask's Nitrogen level (NO_3^--N) using an initial NO_3^- concentration of 1.17×10^{-2} M (4 x $NaNO_3$ in BBM). All calculations are displayed in Table B.4. Additionally, commercial fertilizer was purchased and medium was prepared according to Bold's recipe to evaluate the effectiveness of these non-research grade nutrients. An additional two trios of flasks were prepared with commercial fertilizer created as BBM, leaving out Ethylene Diamine Tetra-acetic Acid (EDTA) in one set of flasks to determine if the free trace metals in the culture medium become toxic to the algal cultures. The basic configuration of flasks is depicted below:

Flask #	Experiment 4 (w/ *x* as "N")	Experiment 5 (w/ *x* as "N")
A1-3	BBM w/ $NaNO_3$	BBM w/ $NaNO_3$
B1-3	BBM w/ $[NH_4]_2SO_4$	BBM w/ $[NH_4]_2SO_4$
C1-3	BBM w/ NH_4NO_3	BBM w/ NH_4NO_3
D1-3	BBM w/ ACS Urea	Comm. Fert. w/ Prilled Urea
E1-3	Comm. Fert. w/ EDTA	Comm. Fert. w/ Autoclave
F1-3	Comm. Fert. w/out EDTA	Comm. Fert. w/out Autoclave

Experiment 6. Algal growth under varying photoperiods.

Erlenmeyer flasks (n=3) were prepared as per the standard experimental conditions listed in Table 3.1 and exposed to light according to their respective

photoperiod requirement (n=4), creating a total population of N=12 flasks. Air was provided to each culture at a rate of 4720 mL/min and CO_2 was added in-line at 190 mL/min (4%). Stoppers were removed and agitation was halted once per day to measure A550. Culture flasks were placed in covered light-tight boxes during the dark phase of the photoperiod. Parameter configuration for Experiment 6 is listed in Table B.5, Appendix B. The basic nomenclature of the configuration is depicted below:

Flask #	Photoperiod (Light:Dark in Hours)
A1-3	24:0
B1-3	18:6
C1-3	12:12
D1-3	6:18

Experiment 7. Algal growth under varying CO_2 concentrations.

Erlenmeyer flasks (n=3) were prepared for each CO_2-in-air concentration (n=10) bringing a total population of N=30 flasks for the experiment. Experimental conditions remain as per Table 3.1, with the exception of CO_2-in-air concentrations. Air was provided to the culture at varying rates, depending on the percentage of CO_2-in-air. Actual flow rates for both CO_2 and air are depicted in Table B.6 and were maintained through the use of a Flow Meter (Cole Parmer 3-N-1, Model # - PMR6-010001, S/N-241379-1). Stoppers were removed and agitation was halted once per day to measure A550. Additionally, pH of the cultures was monitored to track the health status of the algal suspension, and adjusted to ~ 6.6 to ensure growth could be maintained. Culture nomenclature is depicted below:

Flask #		Experiment 7	
A1-3	Ambient (0.04%)	4% (Control)	4% (Control)
B1-3	4% (Control)	10%	25%
C1-3	50%	15%	30%
D1-3	100%	20%	35%
E1-3	N/A	N/A	N/A

IV. Results and Analysis

Introduction.

The results from these experiments offer insight into the effects that certain environmental parameters have on algal growth and productivity. The results were analyzed through the inspection of a variety of calculated or tabulated parameters; these parameters include growth rates, concentration of biomass, absorbance of the algal suspension, and dry weight accumulation. While growth rates and cell concentration are important end-state parameters for analyzing the amount of biomass that will accumulate in a certain period of time and overall productivity of any photo-bioreactor (PBR), absorbance and dry weight analysis are better indicators of growth in this thesis as they represent the actual raw data that was analyzed. However, dry weight was not an end-state method of analysis for any of the aforementioned experiments. Therefore, all analyses were conducted using algal cell suspension absorbance at 550nm. Correlation equations relating Absorbance to Cell Concentration were created each time a new culture of *C. vulgaris* was generated from an agar slant. The regression equations are used to make qualitative assertions and estimations regarding the productivity of a PBR for different volumes and relate to the accumulation of biomass over time. The curves are displayed below as appropriate. Spreadsheet data for each curve is located in Appendix A, Tables A.1 – A.3.

To determine significant differences in growth between cultures under different environmental conditions, the Kruskal-Wallis test with Dunn's Post-Test was performed for each experiment in order to compare the non-parametric data across the various

groups observed in these experiments. Using the Dunn's Post-Test, I was able to determine when significant differences in culture growth occurred in time. These tests were used because the measurement variable (absorbance) does not meet the normality assumption of a one-way ANOVA. There are several additional assumptions built into the test; we must assume that all are random samples from their respective populations, there is independence within each sample, and an observed mutual independence among all samples. The resulting test statistic *K* is then compared to the Chi Square (X^2) statistic at the given *N-1* degrees of freedom and the appropriate confidence level (always α=0.05). If the *K* value calculated is larger than the X^2 value, or if the P-value is less than 0.05, then there is a significant difference in the population and one or more of the groups has performed differently under the given environmental conditions. To calculate the appropriate *K* value, I used the following formulas:

$$K = (N-1)\frac{\sum_{i=1}^{g} n_i(\bar{r}_{i\cdot} - \bar{r})^2}{\sum_{i=1}^{g}\sum_{j=1}^{n_i}(r_{ij} - \bar{r})^2}$$

$$\bar{r}_{i\cdot} = \frac{\sum_{j=1}^{n_i} r_{ij}}{n_i}$$

$$\bar{r} = \tfrac{1}{2}(N+1)$$

where *N* is the total number of observations across all groups, n_i is the number of observations in group *i*, and r_{ij} is the rank among all observations of observation *j* from group *i*. r_{bar} is the average of all r_{ij}.

In each experiment the null hypothesis states that each culture performs the same under a variety of environmental conditions. The alternative hypothesis for each experiment states that the cultures perform differently under a given variety of

environmental conditions. Excel Spreadsheet data and Graphpad Prism® version 5 spreadsheet data for each experiment is included with the accompanying disk.

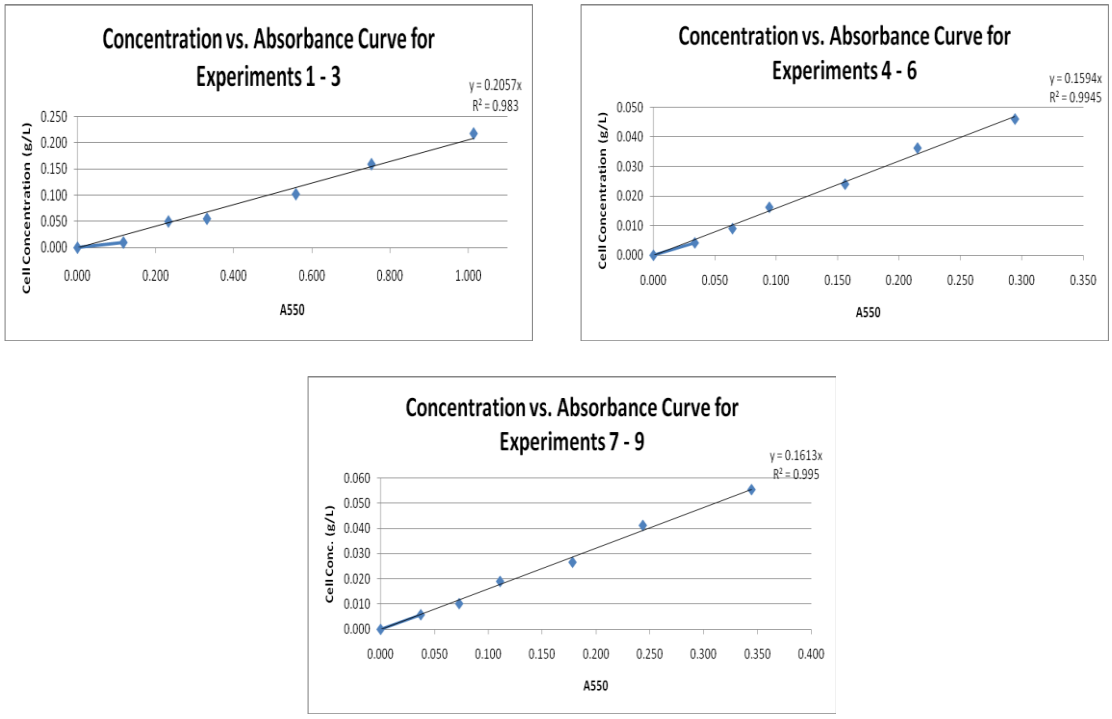


Figure 4.1 – 4.3: *C. vulgaris* Standard Curves used in all experiments, relating Absorbance of the algal suspension at 550 nm to its respective Cell Concentration (g/L).

To determine the validity of each experiment, control cultures were prepared and cultivated. As mentioned before, control cultures consisted of *C. vulgaris* inoculated in BBM with 4 x $NaNO_3$ and D.I. water. Each control was bubbled with a 4% CO_2-in-air mixture, exposed to continuous illumination, and provided with 40 μmol m^{-2} s^{-1} irradiance. A 95% confidence interval was prepared to compare the control culture growth rates. The results are included in the table below:

***Chlorella vulgaris* Control Comparison**											
	Exp 1	**Exp 2**	**Exp 3**	**Exp 4**	**Exp 5**	**Exp 6**	**Exp 7**	**Mean**	**Std Dev**	**Error**	**95% C.I. (mean ±)**
Growth Rate (k) (day^{-1})	0.6381	0.5042	0.6051	0.5380	0.4613	0.6676	0.5966	0.5730	0.074423	0.028129	0.068832614
Doubling time (T_d) (days)	0.9206	0.7274	0.8729	0.7762	0.6654	0.9632	0.8608	0.8266	0.10737	0.040582	0.099304471
Doublings/day (# of doublings)	1.0862	1.3748	1.1456	1.2883	1.5028	1.0382	1.1618	1.2282	0.167327	0.063244	0.15475724
Days in Exp. Growth	Day 2-4	Day 1-6	Day 0-5	Day 0-6	Day 0-6	Day 2-7	Day 0-5				
C.I. Formula: $x_{bar} \pm t_{\alpha/2}\,(s/\sqrt{n})$											
n=7											
$t_{\alpha/2(.025)}$ = 2.447 (based on 6 degrees of freedom; n-1) (Pearson, 1966)											

Table 4.1: Productivity data for Experimental Controls.

Growth rates for the control across all experiments were 0.5730 ± 0.069 day^{-1} (or [0.504 : 0.642 day^{-1}]). Thus, I am 95% confident that the true value of the growth rate for a *C. vulgaris* culture grown under the environmental conditions listed above lies somewhere between 0.504 day^{-1} and 0.642 day^{-1}. This appears to be consistent with available literature surrounding the given environmental conditions. While growth rates for any algal culture will vary dramatically with changes in temperature, irradiance, and nutrient availability, the values observed here appear to fall within the expected range of values. For instance, Illman et al. observed growth rates between 0.43 and 0.99 day^{-1} for similar environmental conditions (25 ° C, 25 μmol m^{-2} s^{-1} irradiance, and 5% CO_2-in-air) (Illman et al., 2000). Hsieh and Wu observed values ranging from 0.8592 – 1.416 when varying the nitrogen type and concentration, but with increased irradiance (600 μmol m^{-2} s^{-1}, and 30 ° C) (Hsieh and Wu, 2009). Others have observed growth of 1.10 day^{-1} (Sobczuk et al., 2008), and 0.96 day^{-1} (Mandalam and Palsson, 1997). Perhaps most interesting are the results observed by Scragg et al. They observed bi-phasic growth rates in the *C. vulgaris* culture; initially the alga grew at 0.69 day^{-1} for four days but then

growth retracted to 0.12 day^{-1}. Additionally, they found that the location of the algal culture impacted its growth rate. Growth in a tubular PBR occurred at 0.40 day^{-1}. However, they observed increased growth rates of 0.99 day^{-1} when cultivation of the alga occurred in 2 L Erlenmeyer flasks (Scragg et al., 2002). In short, several environmental parameters play a role in determining the growth rate for the *C. vulgaris* cultures; irradiance, medium composition, and temperature are the primary parameters affecting growth. While it is difficult to compare control results from this experiment with what others have done, it is not difficult to conclude from my results that growth here in these experiments under these conditions was valid and repeatable.

Experiment 1.

All water varieties supported algal growth. Among the water types investigated, D.I. water supported the best growth with maximum biomass yield of 0.339 g/L by Day 6, the last day of the experiment.

Cell Concentration (g/L)				
Day	**DI Water**	**TW Charcoal Autoclave**	**TW w/ Charcoal**	**Tap Water**
0	0.053	0.055	0.060	0.049
1	0.052	0.051	0.051	0.043
2	0.084	0.082	0.094	0.083
3	0.215	0.166	0.219	0.212
4	0.301	0.260	0.274	0.273
5	0.332	0.268	0.320	0.310
6	0.339	0.251	0.322	0.329

Table 4.2: Biomass Yield per Culture for Experiment 1.

Growth of *C. vulgaris* in D.I. water was significantly greater than that grown using autoclaved charcoal filtered (CF) tap water by Day 3, with significantly better growth over all species by Day 4. Kruskal-Wallis One-way ANOVA with Dunn's multi-

comparison post-test identified growth among the species grown in non-autoclaved tap water and regular tap water versus the control D.I. water as not significantly different on Day 5 or Day 6; however, there was great variance in A550 measurements for both water types (as depicted in Figure 4.5) suggesting that repeatability among experiments may be difficult. While growth did occur with autoclaved CF tap water, it peaked by Day 5 with a reduction in biomass yield by Day 6 to 0.251 g/L, a 7% reduction in biomass versus the previous day and 26% lower yield than the top performing D.I. water culture. The raw data supporting these conclusions is located in Appendix C, and is listed for each culture below:

Growth of *Chlorella vulgaris* during Experiment 1				
	D.I. Water	**CF Water w/ Autoclave**	**CF Water w/out Autoclave**	**Tap Water**
Growth Rate (day^{-1})	0.638	0.577	0.535	0.595
Doublings / day	0.920	0.832	0.772	0.859
Doubling Time (days)	1.086	1.201	1.296	1.164
Max Cell Concentration (g/L)	0.339	0.268	0.322	0.329

Table 4.3: Growth data from Experiment 1.

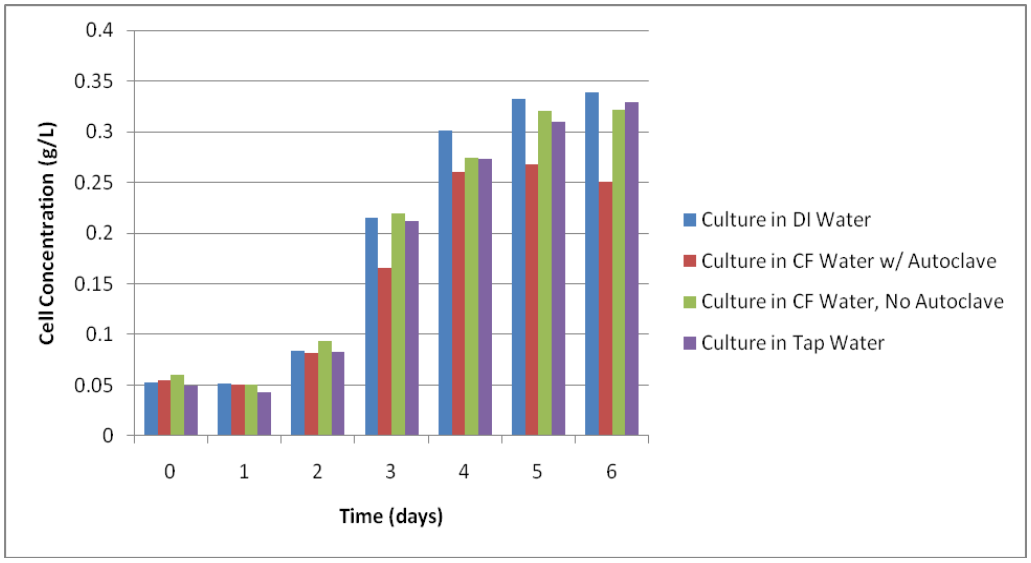
During Experiment 2, growth was again investigated for *C. vulgaris* grown in CF Water with and without autoclave to determine the repeatability of the first experiment. These two culture conditions were repeated because the alga grown in both media configurations performed poorly and it is important to quantify with some accuracy the capability of regular filtered tap water to support algal growth, since D.I. water is logistically burdensome. Second, during Experiment 1 the algae in the autoclaved media solutions exhibited significant cell adherence to the flask bottom, presumably caused by flocculation. There are several potential causes of flocculation in algal cultures; excess abundance of Ca^{2+} and Mg^{2+} ions, an elevated temperature in the media solution (such as

would occur in an autoclave) causing $CaCO_3$ to precipitate, and a lack of nutrients leading to diminished growth rates and eventual attractive charges forming between algal cells causing conglomeration and gravitational settling. All processes will be discussed in Section 5 of this document. Before continuing with the experiments, it was important to repeat the process to determine if the occurrence in Experiment 1 was random or not.

Statistically, growth during Experiment 2 was significantly greater than that observed in Experiment 1. Using the Kruskal-Wallis One-Way ANOVA, I compared the cultures from both experiments to determine significance. With an overall P value of < 0.0001, by Day 1 the cultures from Experiment 2 "with" and "without" Autoclave had outperformed both similar cultures from Experiment 1. More importantly, by Day 2 the autoclaved culture from Experiment 2 was growing at a greater rate than the non-autoclaved culture from Experiment 2 in a statistically significant manner (P value < 0.05). This was the exact opposite result of that observed in Experiment 1 where it was determined through Kruskal-Wallis One-Way ANOVA that growth of the non-autoclaved culture had outperformed the autoclaved culture in a statistically significant manner (P value < 0.05).

Growth of *Chlorella vulgaris* during Experiment 2		
	CF Water w/ Autoclave	**CF Water w/out Autoclave**
Growth Rate (day^{-1})	0.401	0.290
Doublings / day	0.578	0.419
Doubling Time (days)	1.731	2.387
Max Cell Concentration (g/L)	0.840	0.585

Table 4.4: Growth data from Experiment 2 for the cultures that can compare to similar cultures in Experiment 1.



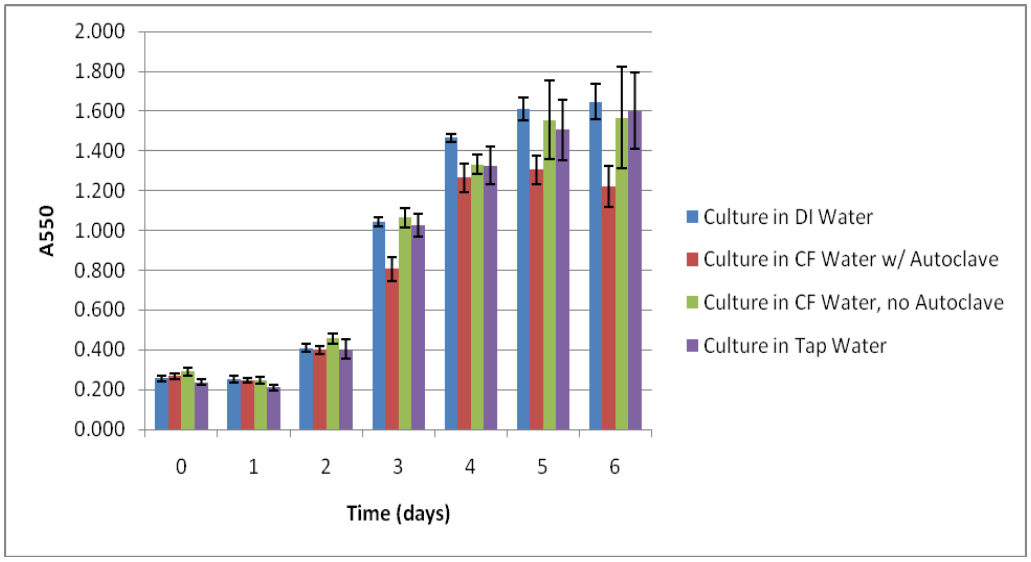


Figure 4.4 and Figure 4.5: Results from Experiment 1 depicting *C. vulgaris* yield through growth in BBM with 4 x $NaNO_3$ using different water types. Figure 4.4 reflects cell concentration changes with time while Figure 4.5 reflects A550 changes with time, and the error associated with each measurement. Note the performance of the culture grown in the media containing autoclaved Charcoal Filtered Tap Water.

Experiment 2.

Using non-autoclaved CF tap water during experiment 2, cultures were grown on an alternate nutrient type (Scott's Peters® Excel® Cal-Mag 15-5-15) and compared with

the growth of the *C. vulgaris* control (BBM w/ 4 x $NaNO_3$ in D.I. water). As in Experiment 1, the control outperformed all cultures grown in CF tap water with maximum biomass yield of 1.009 g/L occurring on Day 8. The algae growing in 1 g/L Cal-Mag solution peaked at 0.275 g/L by Day 6 with decline on both Day 7 and Day 8. Algae grown using 2 g/L and 5 g/L exhibited a seven-day lag-phase before any growth occurred. This did not occur with the 1 g/L algae / Cal-Mag solution. Once growth began in the lagging cultures, the algae appeared to grow with similar rates as the control. However, based on the results indicated in Table 4.5 with growth rates depicted in Table 4.6, there seems to be an inverse correlation between growth rate and biomass yield for the Cal-Mag cultures. Additionally, from these results it is evident that greater biomass yield results from a greater concentration of Cal-Mag nutrient solution. However, as is indicated in the Table 4.6, growth rate suffered accordingly.

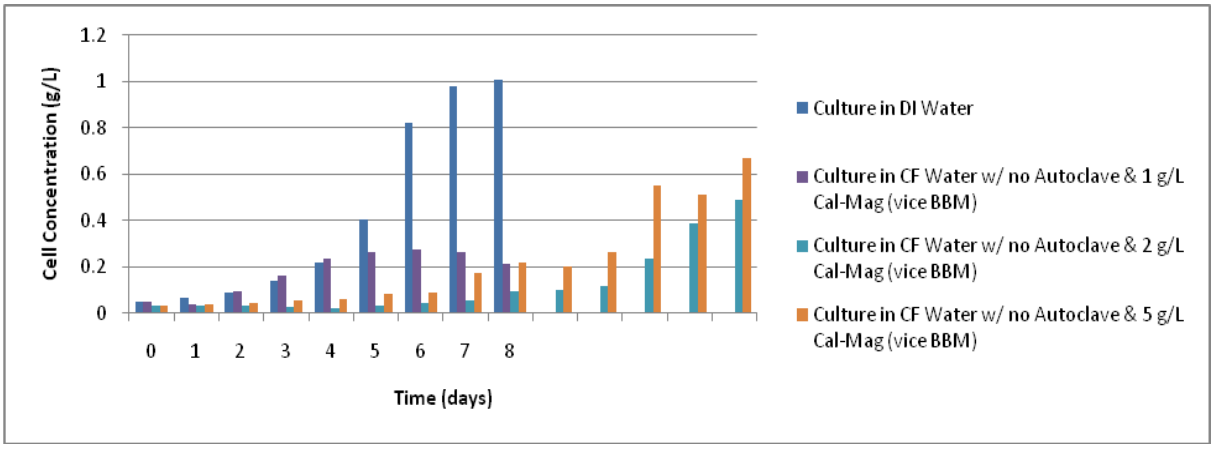
Cell Concentration (g/L)				
Day	**DI Water**	**TW Charcoal Cal-Mag (1 g/L)**	**TW Charcoal Cal-Mag (2 g/L)**	**TW Charcoal Cal-Mag (5 g/L)**
0	0.048	0.048	0.032	0.034
1	0.066	0.041	0.033	0.040
2	0.091	0.095	0.030	0.043
3	0.140	0.165	0.026	0.054
4	0.217	0.233	0.022	0.062
5	0.404	0.264	0.032	0.083
6	0.821	0.275	0.043	0.088
7	0.982	0.265	0.053	0.173
8	1.009	0.214	0.095	0.216
9	N/A	N/A	0.099	0.199
10	N/A	N/A	0.117	0.266
11	N/A	N/A	0.235	0.551
12	N/A	N/A	0.385	0.514
13	N/A	N/A	0.487	0.668

Table 4.5: Biomass Yield per Culture for Experiment 2.

Growth of *Chlorella vulgaris* during Experiment 2				
	BBM w/ 4x$NaNO_3$	**1 g/L Cal-Mag**	**2 g/L Cal-Mag**	**5 g/L Cal-Mag**
Growth Rate (day^{-1})	0.504	0.579	0.475	0.307
Doublings / day	0.727	0.836	0.686	0.443
Doubling Time (days)	1.375	1.197	1.458	2.258
Max Cell Concentration (g/L)	1.009	0.275	0.487	0.668

Table 4.6: Growth data for Experiment 2.

Growth in the control culture was significantly greater than both the 2 g/L and 5 g/L algae / Cal-Mag solutions by Day 1, which makes sense because both cultures exhibited a seven-day lag phase. Growth of the control was not significantly greater than the 1 g/L algal / Cal-Mag solution until Day 5. Error bars in Figure 4.7 below indicate a lack of significance between the 5 g/L algal / Cal-Mag solution and the control at Day 6 of each's respective growth phase. However, there is significant variance in the 5 g/L data resulting from one Erlenmeyer flask outperforming the other two flasks by triple their absorbances at 550 nm throughout their growth phases. Direct correlation with the control could not occur as it had reached the stationary and death phase of its life cycle by the time the 2 g/L and 5 g/L algae / Cal-Mag solutions had exited their lag phases. Therefore, qualitative correlation was used to infer performance characteristics. The raw data obtained in Experiment 2 is located in Appendix D.



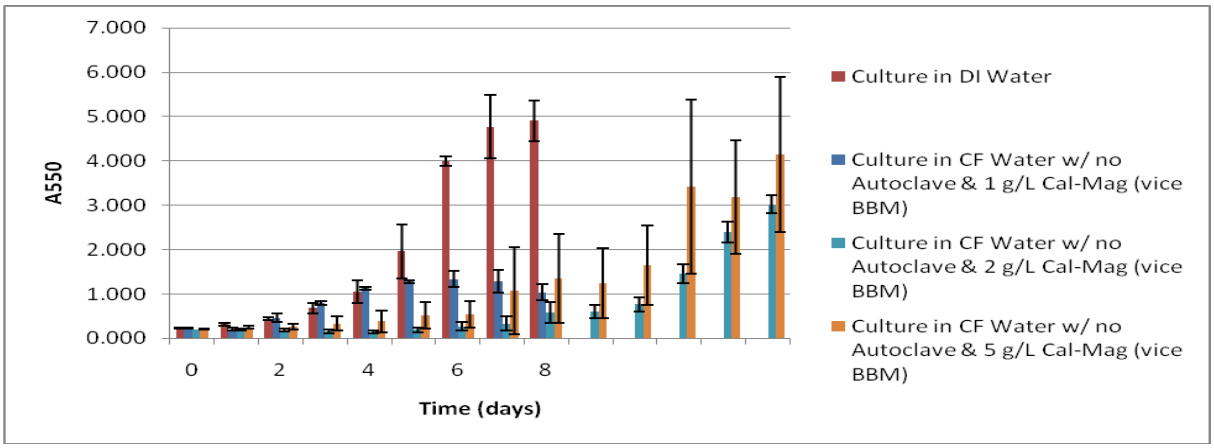


Figure 4.6 and Figure 4.7. Results from Experiment 2 depicting *C. vulgaris* yield through growth in BBM with 4 x $NaNO_3$ using the water type indicating best performance in Experiment 1. Figure 4.6 reflects the changes in cell concentration with time, while Figure 4.7 reflects absorbance changes for each culture, and the error associated with each measurement. Note the performance of the culture grown in media based on 1 g/L Cal-Mag (vice BBM). Also, note that the Control (using DI Water) performed best in both Experiments 1 & 2. Results indicate significant lag time associated with both the 2 g/L and 5 g/L Cal-Mag cultures.

Experiment 3.

The results of this experiment indicate that the most dilute algae culture demonstrated the greatest growth rate over time (Table 4.8) and potentially yielded the greatest biomass. While the culture that began with an $A550_0$ of 0.050 produced the most

biomass over the 18 day experiment (3.473 g/L), it is clear that the more dilute culture with an $A550_0$ of 0.025 would have exceeded that value, considering its growth rate.

Cell Concentration (g/L)				
Day	DI (2mL)	DI (1mL)	DI (0.5mL)	DI (0.25mL)
0	0.040	0.021	0.011	0.006
1	0.09	0.050	0.026	0.014
2	0.188	0.126	0.065	0.042
3	0.307	0.208	0.151	0.095
4	0.381	0.296	0.265	0.206
5	0.824	0.609	0.547	0.471
6	0.899	0.707	0.668	0.621
7	1.101	0.938	0.909	0.847
8	1.150	1.003	0.972	0.875
9	1.258	1.055	1.087	1.037
10	1.308	1.166	1.155	1.127
11	1.366	1.224	1.251	1.224
12	1.458	1.335	1.374	1.343
13	1.557	1.429	1.512	1.486
14	1.774	1.628	1.608	1.628
15	1.947	2.116	2.115	1.977
16	2.181	2.298	2.590	2.308
17	2.313	2.699	2.957	2.610
18	2.716	2.937	3.473	3.268

Table 4.7: Biomass Yield per Culture for Experiment 3.

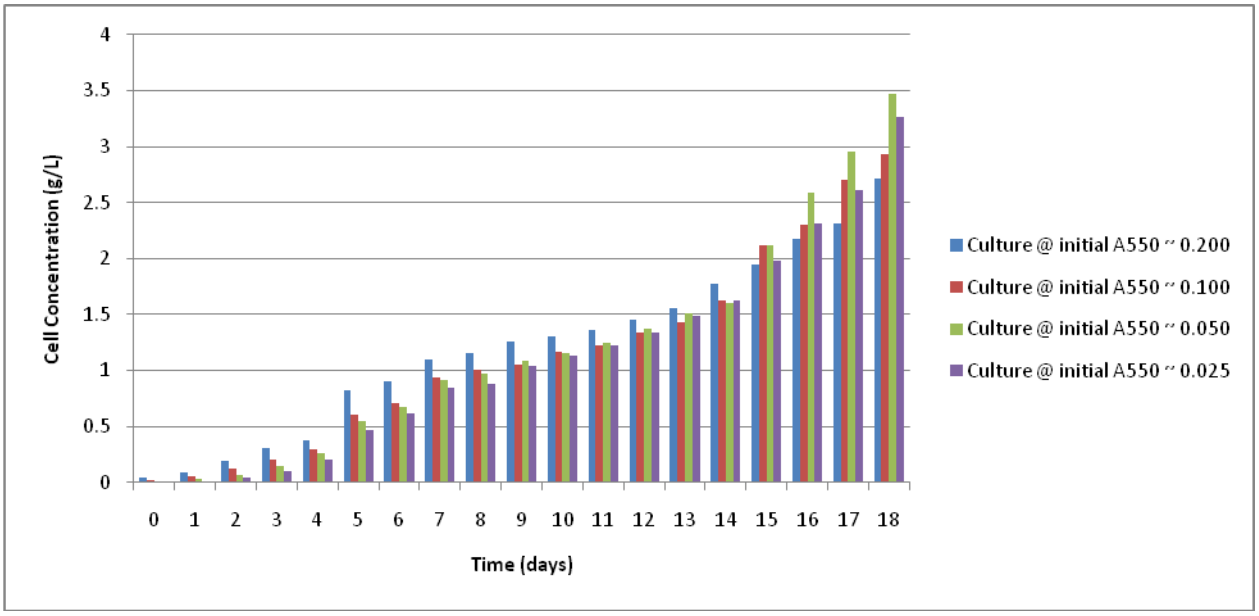
As can be seen in Figures 4.8 and 4.9 below or in Table 4.7 above, the more concentrated cultures with $A550_0$ of 0.200 and 0.100 exhibited rapid growth and yield exceeding that in the more dilute cultures with $A550_0$ of 0.050 and 0.025 in the early portion of the experiment, through Day 9. But, by Day 10 and into Day 11 the culture growth differences were not significant. In fact, through Day 9, all cultures are statistically different in their growth with an overall P value of 0.0004 for the experiment and with significant differences in Dunn's post-test comparisons between cultures. However, by Day 10, growth in the cultures with $A550_0$ of 0.100 and 0.050 caught up to

the culture with $A550_0$ of 0.200. This is evidenced in the data. The Day 10 Kruskal-Wallis growth comparisons indicated there was a significant difference in some of the cultures (P value = 0.0134). Follow-on post-test comparisons prove that there is no statistical difference between the $A550_0$ cultures with 0.200, 0.100, and 0.050 but growth in the 0.025 culture is statistically different. By Day 11, growth of all cultures was statistically the same (overall P value = 0.0769). As mentioned in the introduction to this section, all Prism® data is contained in the accompanying CD.

The results of this experiment indicate that the more dilute cultures have a greater capacity for biomass production over time. However, as depicted here, this productivity comes only after > 18 days of culture growth. If rapid biomass production is the goal, cultures with $A550_0$ of 0.200 are sufficient. As can be seen in Table 4.7 above, cell concentrations of > 1.1 g/L are possible within seven days using an $A550_0$ of 0.200. Another 3 days of growth are required for the more dilute cultures to reach ~ 1.1 g/L concentration.

Growth of *Chlorella vulgaris* during Experiment 3				
	$A550_0$ ~ 0.200	**$A550_0$ ~ 0.100**	**$A550_0$ ~ 0.050**	**$A550_0$ ~ 0.025**
Growth Rate (day^{-1})	0.605	0.673	0.781	0.873
Doublings / day	0.873	0.972	1.127	1.259
Doubling Time (days)	1.146	1.029	0.887	0.794
Max Cell Concentration (g/L)	2.716	2.937	3.473	3.268

Table 4.8: Growth data for Experiment 3.



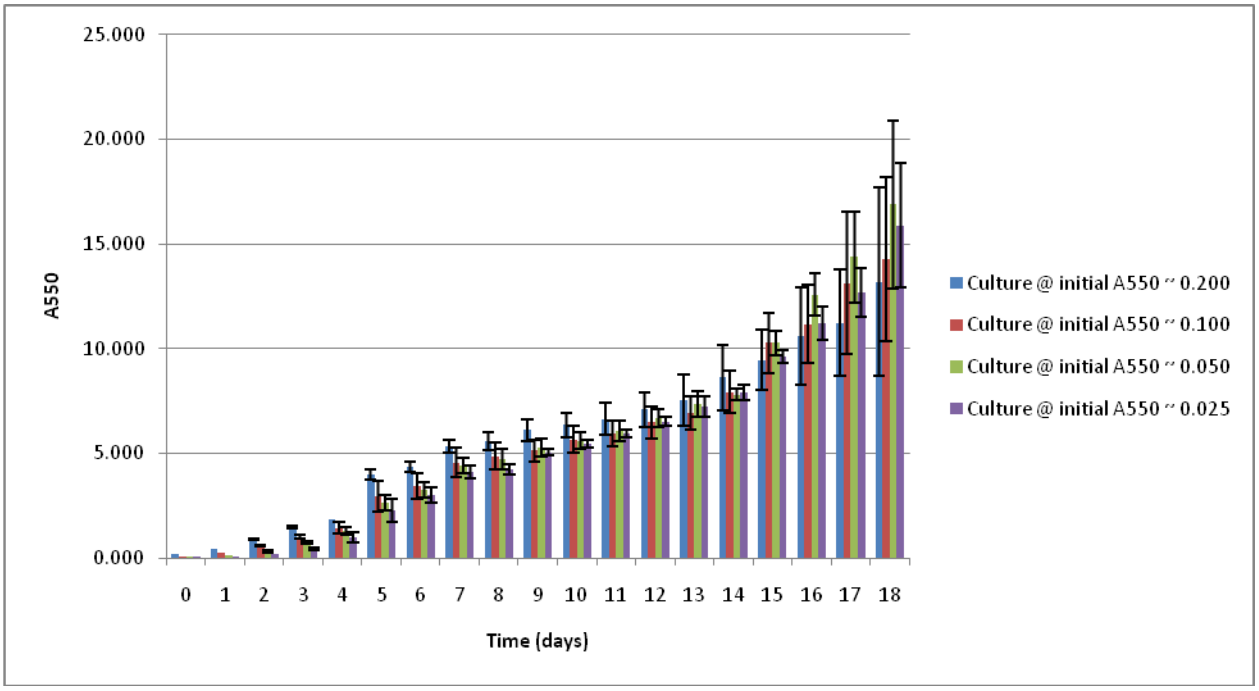


Figure 4.8 and Figure 4.9: Results from Experiment 3. Figure 4.8 reflects cell concentration changes with time, while Figure 4.9 reflects changes in the culture absorbance with time, and the error associated with each measurement. Note the non-significant differences in culture absorbances as they progress through the exponential growth phase of their life-span. Note that the dilute cultures (A550 of 0.050 and 0.025) exhibited lower growth rates initially compared to the more concentrated cultures (A550 of 0.200 and 0.100), but higher rates of growth beginning at Day13.

Experiment 4.

The results displayed in Table 4.10 indicate that the greatest growth occurred in the culture using Urea as "N" and in the cultures using commercial fertilizer made up as BBM with 4 x $NaNO_3$, each doubling approximately once per day. The algal culture using BBM with Urea as "N" showed the greatest rate of growth and reached its maximum biomass concentration by Day 7 with 2.003 g/L. However, by Day 10 that same culture had declined in biomass concentration to 1.634 g/L indicating that the rapid early growth of the culture consumed a large portion of the most reduced "N" form (NH_3) creating a "N" limitation in the last few days of the experiment; thus, continued growth could not be sustained. Conversely, the algal cultures grown on Commercial Fertilizer made up as BBM with 4 x $NaNO_3$ exhibited no decline and showed continued growth up to a maximum biomass concentration of 2.322 g/L (CF with EDTA).

Cell Concentration (g/L)						
Day	**$NaNO_3$**	**NH_4SO_4**	**NH_4NO_3**	**Urea**	**CF w/ EDTA STEM**	**CF w/out EDTA STEM**
0	0.030	0.031	0.032	0.034	0.033	0.033
1	0.073	0.076	0.074	0.069	0.076	0.080
2	0.123	0.134	0.115	0.187	0.212	0.243
3	0.251	0.179	0.154	0.360	0.457	0.549
4	0.470	0.214	0.156	0.739	0.750	0.731
5	0.584	0.195	0.152	1.134	1.001	1.036
6	0.757	0.179	0.145	1.720	1.545	1.633
7	1.017	0.238	0.205	2.003	1.964	1.775
8	1.103	0.144	0.182	1.758	1.629	1.413
9	1.054	0.130	0.178	1.494	1.994	1.833
10	1.244	0.129	0.182	1.634	2.322	1.716

Table 4.9: Biomass Yield per Culture for Experiment 4.

Except for the cultures grown using $(NH_4)_2SO_4$ and NH_4NO_3 as "N", all cultures displayed better growth than the control in a statistically significant manner over the

course of the experiment. This occurred during different times in the experiment, but overall between Day 3 and Day 6. With the exception of the control, by Day 2 all cultures were significantly different (P value < 0.0001) compared to the cultures grown using $(NH_4)_2SO_4$ and NH_4NO_3 as "N". The control culture broke away from the $(NH_4)_2SO_4$ and NH_4NO_3 cultures by Day 3. In fact, the cultures grown using those two media demonstrated moderate initial growth but eventual decline after Day 4, yielding only 0.238 g/L and 0.205 g/L biomass concentration respectively. This is discussed in the following paragraph.

Growth of *Chlorella vulgaris* during Experiment 4						
	$NaNO_3$	**$(NH_4)_2SO_4$**	**NH_4NO_3**	**Urea (ACS grade)**	**Comm. Fert. w/ EDTA**	**Comm. Fert. w/out EDTA**
Growth Rate (day^{-1})	0.538	0.482	0.396	0.654	0.641	0.650
Doublings / day	0.776	0.696	0.571	0.943	0.924	0.938
Doubling Time (days)	1.288	1.435	1.750	1.059	1.081	1.065
Max Cell Concentration (g/L)	1.244	0.238	0.205	2.003	2.322	1.833

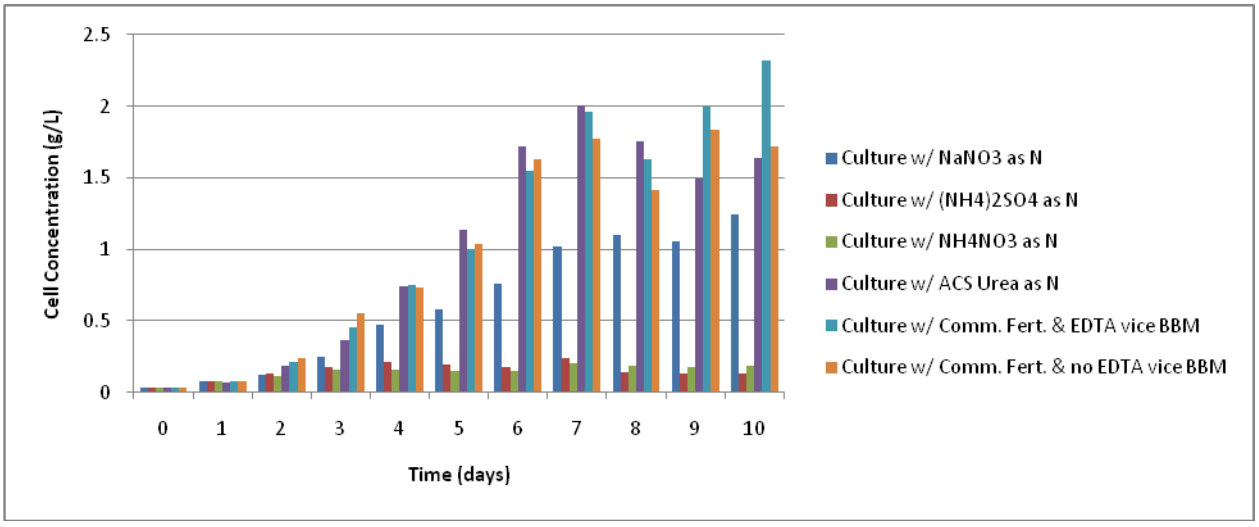
Table 4.10: Growth data for Experiment 4.

Experiment 4 relied on the natural buffering capacity of the *C. vulgaris* in a water-based system. Therefore, pH was not adjusted daily to ~ 6.6, the value suggested in Bold's recipe (Bold, 1949; Bischoff and Bold, 1963). This provided information on the robustness of this particular alga to sustain itself over a wide pH range. pH was not greatly affected in the control culture, or in the cultures grown using Urea, Commercial Fertilizer with EDTA, or Commercial Fertilizer without EDTA, indicating

Cell Concentration (g/L) and Culture pH Experiment 4				
Day	**$(NH_4)_2SO_4$**	**pH**	**NH_4NO_3**	**pH**
0	0.031	6.6	0.032	6.6
1	0.076	6.2	0.074	6.4
2	0.134	5.9	0.115	6.15
3	0.179	5.2	0.154	5.5
4	0.214	3.5	0.156	4
5	0.195	3.5	0.152	4.1
6	0.179	3.6	0.145	4.3
7	0.238	3.7	0.205	4.4
8	0.144	3.6	0.182	4.3
9	0.130	3.7	0.178	4.3
10	0.129	4.3	0.182	4.36

Table 4.11: Cell Concentrations and associated culture pH for Experiment 4.

that in those media solutions the natural buffering capacity was sufficient to sustain the alga's life. Daily pH values for the two underperforming algal solutions are depicted in Table 4.11. As can be seen in Table 4.11, as pH passed below 5, the cultures were adversely affected. While some growth occurs as the culture pH passes through 5 and approaches 4, it is stagnant and at best minimal. Neither the culture using $(NH_4)_2SO_4$ nor NH_4NO_3 as "N" exhibited growth at their ultimate pHs. In fact, cell concentrations for each were approximately $1/20^{th}$ of that achieved in the cultures using other forms of "N". All data supporting these findings is located in Appendix F.



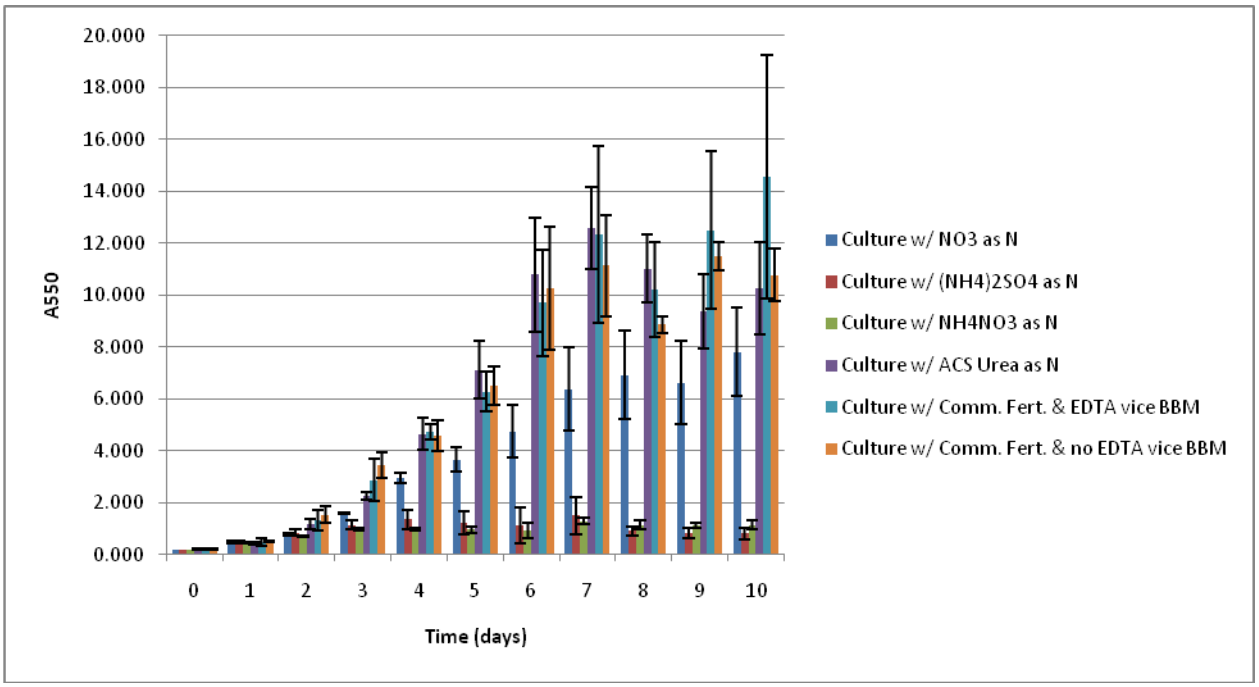


Figure 4.10 and Figure 4.11: Results from Experiment 4. Figure 4.10 reflects changes in cell concentration with time, while Figure 4.11 reflects changes in culture absorbance with time, and the error associated with each measurement. Note the growth for the control culture grown on BBM with $NaNO_3$ as "N" compared to the growth for the commercial fertilizer blends made up according to the Bold's Basal Medium recipe. Also note the non-growth exhibited by the cultures using $(NH_4)_2SO_4$ and NH_4NO_3 as "N".

Experiment 5.

Growth rates and biomass yield observed during Experiment 5 were lower for the control than observed in Experiment 4, as depicted in Table 4.12 and Table 4.13 below.

Cell Concentration (g/L)						
Day	**$NaNO_3$**	**NH_4SO_4**	**NH_4NO_3**	**Comm. Fert w/ Prilled Urea as "N"**	**CF w/ autoclave**	**CF w/out autoclave**
0	0.037	0.037	0.037	0.037	0.024	0.024
1	0.057	0.062	0.065	0.075	0.052	0.058
2	0.115	0.153	0.139	0.150	0.139	0.132
3	0.212	0.161	0.201	0.226	0.225	0.181
4	0.289	0.241	0.277	0.318	0.419	0.246
5	0.483	0.348	0.394	0.429	0.457	0.345
6	0.589	0.436	0.510	0.535	0.577	0.466

7	0.684	0.518	0.574	0.611	0.682	0.553
8	0.771	0.604	0.628	0.688	0.770	0.622
9	0.776	0.586	0.697	0.693	0.793	0.615
10	0.857	0.660	0.649	0.729	0.744	0.615
11	0.813	0.692	0.734	0.853	0.920	0.692

Table 4.12: Biomass Yield per Culture for Experiment 5.

Additionally, the culture utilizing Non-Autoclaved Commercial Fertilizer performed considerably worse in Experiment 5 than it did during Experiment 4 (culture with Non-Autoclaved Commercial Fertilizer and EDTA). The observed growth rates from Experiment 4 and 5 were 0.641 and 0.494 respectively, and maximum biomass yield was 2.322 g/L and 0.692 g/L respectively.

Growth of *Chlorella vulgaris* during Experiment 5						
	$NaNO_3$	**$(NH_4)_2SO_4$**	**NH_4NO_3**	**Comm. Fert. w/ Prilled Urea as "N"**	**Comm. Fert. w/ Autoclave**	**Comm. Fert, No Autoclave**
Growth Rate (day^{-1})	0.461	0.411	0.437	0.445	0.530	0.494
Doublings / day	0.665	0.593	0.631	0.642	0.765	0.713
Doubling Time (days)	1.503	1.686	1.585	1.557	1.308	1.402
Max Cell Concentration (g/L)	0.857	0.692	0.734	0.853	0.920	0.692

Table 4.13: Growth Data for Experiment 5.

The culture grown during Experiment 5 on Commercial Fertilizer made as BBM with Prilled Urea as "N" did not produce as much biomass as that using American Chemical Society (ACS) grade Urea in Experiment 4 indicating that the Prilled Urea was not of good nutrient quality. Considering the performance of each culture in this experiment, and the fact that all environmental conditions were identical to Experiment 4, it is possible that growth did not occur in the same manner as it did in Experiment 4 due to random biological occurrences and not because of the nutrient type used.

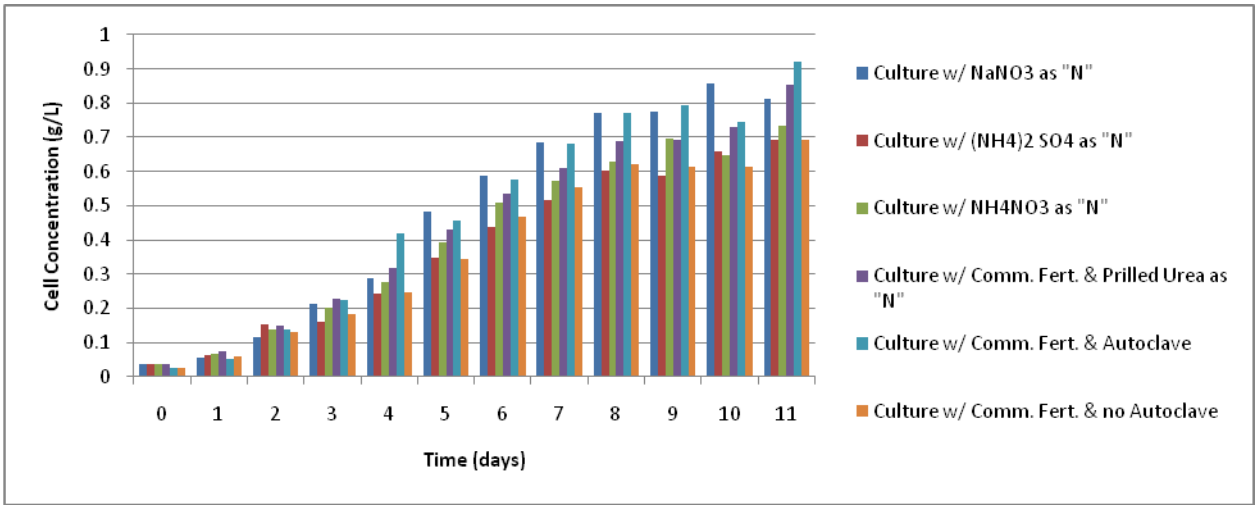
Yet, even with reduced growth rates and yields, there was one interesting finding involving the effect of pH on a culture's viability. During Experiment 5, pH was adjusted every day after A550 measurements were taken. This ensured that the algae continued to grow unimpeded under optimal pH conditions. In fact, referring to Table 4.14, and comparing the values with Table 4.11 in the previous section, one can observe the effect that pH had on this particular alga. By maintaining the pH at ~ 6.6, the alga continued to grow. At Day 4, cell concentrations began to decline in Experiment 4 but continued to grow in Experiment 5 through Day 11. Recalling from before, I reported that by Day 4 pH began to plummet to a region where algal biomass could not be maintained. This was the point where the algae could not effectively buffer themselves in the water based solution. By artificially buffering the algal solutions during Experiment 5 with KOH, cell growth continued and biomass was produced.

Cell Concentration (g/L) and Culture pH Experiment 5				
Day	$(NH_4)_2SO_4$	pH	NH_4NO_3	pH
0	0.037	6.6	0.037	6.6
1	0.062	6.6	0.065	6.6
2	0.153	6.6	0.139	6.6
3	0.161	6.6	0.201	6.6
4	0.241	6.6	0.277	6.6
5	0.348	6.6	0.394	6.6
6	0.436	6.6	0.510	6.6
7	0.518	6.6	0.574	6.6
8	0.604	6.6	0.628	6.6
9	0.586	6.6	0.697	6.6
10	0.660	6.6	0.649	6.6
11	0.692	6.6	0.734	6.6

Table 4.14: Cell Concentrations and associated culture pH for Experiment 5.

Over the course of the experiment, the differences observed in growth and yield among the cultures were not statistically significant. However, there were some differences observed from day-to-day during the experiment. For instance, between Days 3 and 4 the Autoclaved Commercial Fertilizer algae solution outperformed the $(NH_4)_2SO_4$ as "N" solution, NH_4NO_3 as "N" solution, and the Non-Autoclaved Commercial Fertilizer solutions with a P value of 0.0042. But, the statistical differences

disappeared in all subsequent days, indicating that during this experiment, nitrogen type did not matter.



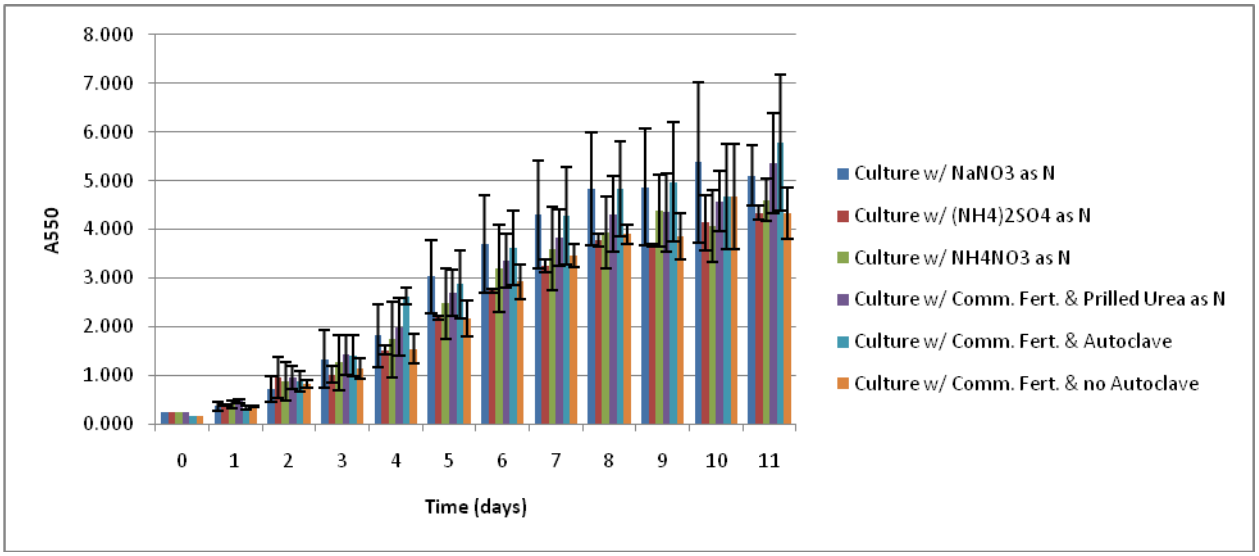


Figure 4.12 and Figure 4.13: Results from Experiment 5. Figure 4.12 reflects changes in cell concentration with time, while Figure 4.13 reflects absorbance changes with each culture, and the error associated with each measurement. Note the growth exhibited by the cultures using $(NH_4)_2SO_4$ and NH_4NO_3 as "N" when pH is adjusted every day to ~ 6.6.

Experiment 6.

Maximum biomass yield of 1.041 g/L occurred in the algal culture exposed to continuous illumination. This value represents a 33% increase in cell concentration over that present in the algal culture exposed to 18 hours of light per day. Cultures exposed to 12 and 6 hours of light per day exhibited negative growth, indicating that biomass was sacrificed by the culture in order to survive in the apparent low-light conditions. Biomass growth for each culture is listed below in Table 4.15 and growth rate data is listed in Table 4.16.

Cell Concentration (g/L)				
Day	**24:0**	**18:6**	**12:12**	**6:18**
0	0.031	0.032	0.032	0.031
1	0.025	0.024	0.023	0.023
2	0.018	0.018	0.018	0.018
3	0.034	0.026	0.017	0.016
4	0.069	0.039	0.015	0.012
5	0.145	0.102	0.016	0.013
6	0.253	0.133	0.016	0.011
7	0.507	0.283	0.016	0.012
8	0.706	0.469	0.017	0.012
9	0.849	0.589	0.017	0.011
10	1.041	0.750	0.021	0.013

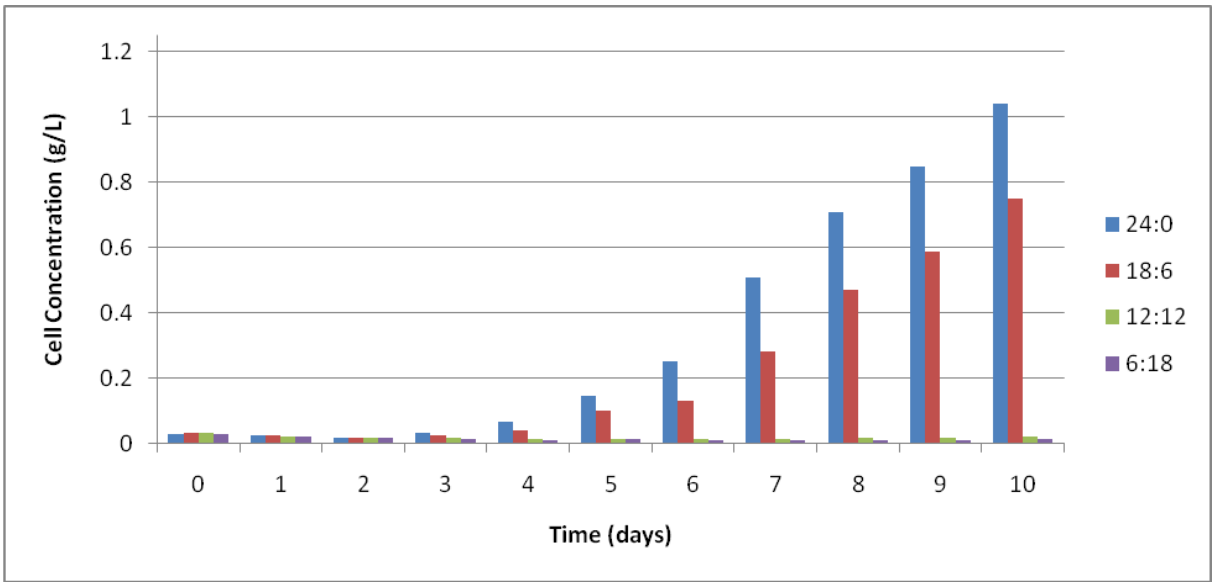
Table 4.15: Biomass Yield per Culture for Experiment 6.

Growth of *Chlorella vulgaris* during Experiment 6				
	24:0	**18:6**	**12:12**	**6:18**
Growth Rate (day^{-1})	0.668	0.551	-0.024	-0.081
Doublings / day	0.963	0.795	-0.034	-0.117
Doubling Time (days)	1.038	1.258	-29.425	-8.548
Max Cell Concentration (g/L)	1.041	0.75	0.032	0.031

Table 4.16: Growth Data for Experiment 6.

From the data gathered in this experiment, it appears that the threshold illumination requirement rests somewhere between the 18 hour and 12-hour per day photoperiod. It is important to note here that the results gathered are indicative only of algal growth under the experimental conditions listed in Section 3. Photoperiod requirements for this algal species may be considerably different under different irradiance conditions and under different temperatures. All data for Experiment 6 can be found in Appendix H.

After an initial two-day lag phase (unknown cause; algal cells were taken from stock culture in exponential growth), both the 24 and 18-hour photoperiod cultures displayed greater growth in a statistically significant manner than the 12 and 6-hour photoperiod cultures (P value < 0.0001). This trend continued over the course of the experiment. However, based on the results of the Kruskal-Wallis test with Dunn's post-test, the difference in growth between the 24-hour and the 18-hour photoperiods was not significant. This is likely a result of one 24-hour photoperiod flask underperforming over the course of the experiment and not likely a result of over-performance by the other two 24-hour photoperiod flasks. This conclusion is based on the growth rates observed among the 24-hour photoperiod flasks as a trio during Experiment 6 (exact same conditions as the control) in comparison with the other experimental controls.



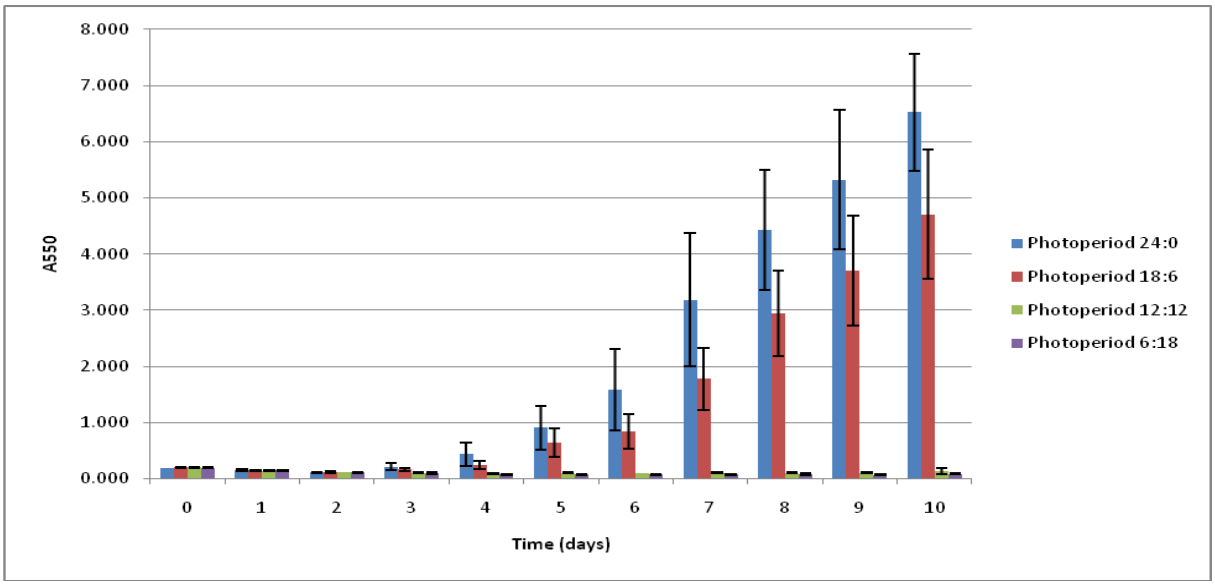


Figure 4.14 and Figure 4.15: Results from Experiment 6. Figure 4.14 reflects the changes in cell concentration with time while Figure 4.15 reflects changes in absorbance for each culture, and the error associated with each measurement. Note the growth for Photoperiods 24:0 and 18:6 indicating that *C. vulgaris* requires at least greater than 12 hours per day of light at irradiance of 40 µmol m^{-2} s^{-1} to support growth.

Experiment 7.

With the exception of the 100% CO_2-in-air mixture, all CO_2-in-air concentrations supported algal growth. Maximum biomass yield was achieved by the control culture (4% CO_2-in-air) with 2.283 g/L biomass by Day 10 of the experiment and can be viewed in the table below.

Cell Concentration (g/L)										
Day	4%	Ambient	10%	15%	20%	25%	30%	35%	50%	100%
0	0.039	0.040	0.040	0.041	0.039	0.034	0.034	0.033	0.043	0.042
1	0.066	0.058	0.080	0.096	0.072	0.069	0.061	0.059	0.054	0.036
2	0.109	0.122	0.107	0.176	0.116	0.116	0.083	0.089	0.065	0.033
3	0.297	0.307	0.317	0.526	0.282	0.272	0.166	0.172	0.094	0.037
4	0.519	0.430	0.410	0.636	0.426	0.397	0.200	0.276	0.111	0.036
5	0.770	0.526	0.825	1.205	0.732	0.576	0.409	0.450	0.126	0.037
6	0.990	0.654	1.134	1.358	1.020	0.734	0.559	0.542	0.102	0.030
7	1.236	0.820	1.201	1.503	0.984	0.837	0.635	0.584	0.129	0.034
8	1.446	0.951	1.422	1.608	1.243	0.910	0.806	0.819	0.145	0.031
9	2.078	1.299	1.575	1.806	1.532	1.083	0.960	0.892	0.268	0.037
10	2.283	1.446	1.677	1.668	1.445	1.217	0.990	0.891	0.381	0.034

Table 4.17: Biomass Yield per Culture for Experiment 7.

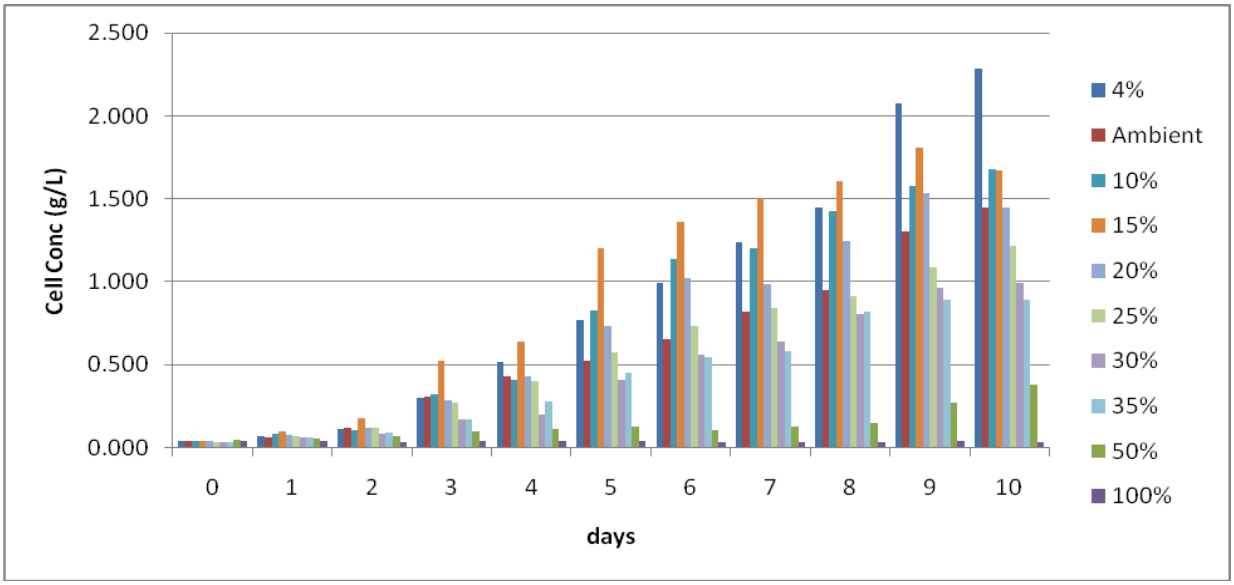
However, the maximum growth rate observed was by the 15% CO_2-in-air culture, with 0.676 day^{-1}, as viewed in Table 4.18 below. Outside of the control culture, biomass yield was greater in this culture than in all others; however, a maximum biomass concentration value of 1.806 g/L was achieved on Day 9 of the experiment with a loss of biomass to 1.668 g/L by Day 10. This represents an 8% reduction of biomass in the 15% CO_2-in-air culture, indicating that the faster growth rate exhibited by the algae reduced the available nutrients in solution to limiting levels. Algal growth and yield represented a direct correlation with CO_2 concentrations up to and including the 15% CO_2-in-air culture. However, the correlation changed to an inverse relationship for all values up to and including the 50% CO_2-in-air culture. There was no growth in the 100% CO_2-in-air cultures; in fact, steady decay of the culture occurred over the course of the ten day experiment.

Growth of *Chlorella vulgaris* during Experiment 7										
	4%	**Ambient**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**50%**	**100%**
Growth Rate (day^{-1})	0.597	0.515	0.605	0.676	0.586	0.566	0.497	0.523	0.215	-0.025
Doublings / day	0.861	0.743	0.873	0.975	0.846	0.816	0.718	0.754	0.310	-0.037
Doubling Time (days)	1.162	1.345	1.145	1.025	1.182	1.225	1.393	1.326	3.224	-27.343
Max Cell Concentration (g/L)	2.283	1.446	1.677	1.806	1.532	1.217	0.990	0.892	0.381	0.042

Table 4.18: Growth data for Experiment 7.

By Day 1 of the experiment, the control culture exhibited growth that was greater than the 100% CO_2-in-air culture in a statistically significant manner, and by Day 2 all cultures from the control up to and including the 25% CO_2-in-air mixture were growing faster and producing more biomass than both the 50% and 100% CO_2-in-air cultures (P value < 0.0001). Interestingly, also by Day 2 the 15% CO_2-in-air culture grew at a faster

rate than all cultures using greater than or equal to 30% CO_2-in-air. Additionally, by Day 5, growth among the 15% CO_2-in-air cultures was statistically greater than the ambient air culture. After looking at the data, it became evident that CO_2-in-air concentrations of between 0.04% (ambient) and 15% supported enhanced algal growth while concentrations between 20% and 50% supported slow growth. 100% CO_2-in-air mixtures did not support algal growth, but instead appeared to be toxic.



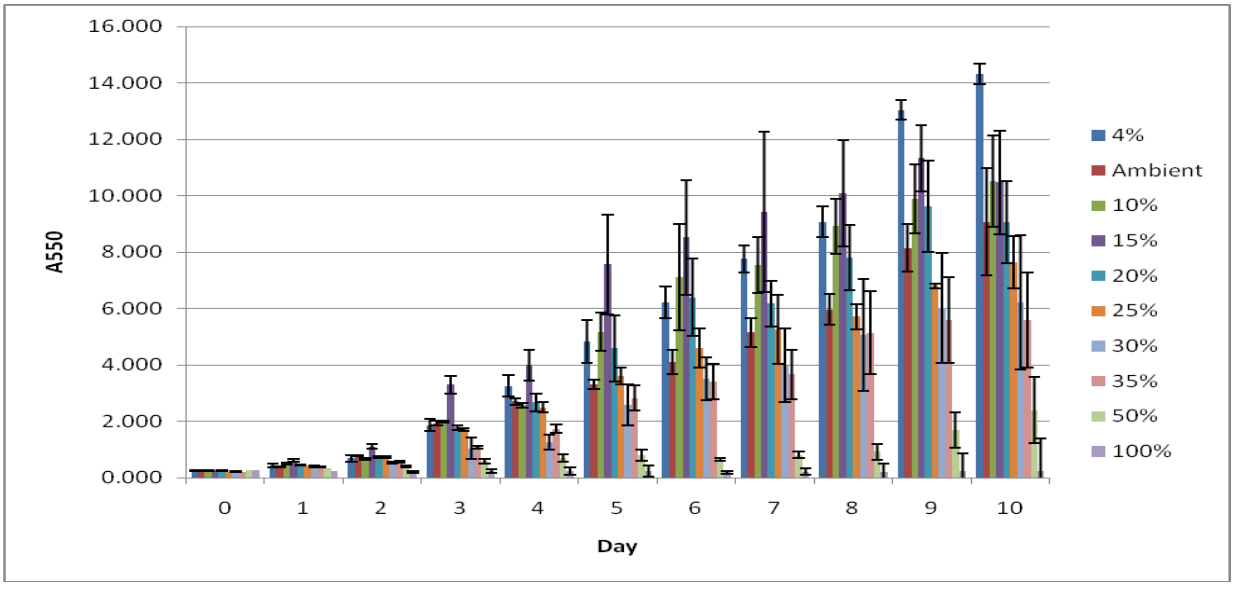


Figure 4.16 and Figure 4.17: Results from Experiment 7. Figure 4.16 reflects changes in cell concentration with time. Figure 4.17 reflects changes in absorbance for each culture with time, and the error associated with each measurement. Note the growth for the control culture outperformed all other cultures in a significant manner by Day 10. With increasing CO_2 concentrations, growth rates appear to decline after 15% and the cultures growing on 100% CO_2 showed no algal growth, indicating that the *C. vulgaris* species cannot tolerate elevated CO_2 concentrations; however, it can tolerate and thrive in the typical percentage of CO_2 in coal-fired power plant flue gas (13-16% CO_2-in-air).

Theoretical CO_2 Sequestration

Although discussed as an analytical method in Section 3 and described in Table A.4 of Appendix A, theoretical calculations for CO_2 sequestration were not included in the Results or Discussion sections of this document because the efficiencies were very low (~ 0.5 %). This indicates that air-lift bubble systems are inadequate when using solution volumes as low as 100 mL in 250 mL Erlenmeyer flasks if reductions in CO_2 are the goal (as would be the case for a power plant attempting to reduce the amount of CO_2 emitted to the atmosphere). This summation is easy to understand, considering the CO_2-in-air flow rate through the solutions of low volume. The bubbles simply do not have enough residence time in the solution before gassing off for the CO_2 to dissolve from the gas to the aqueous phase where they can be used by the algae for photosynthesis. One would expect with larger volume solutions, sequestration efficiencies would increase. Finally, if actual sequestration values are desired by the reader, one could easily determine them using the data available in Appendices C – I and the example calculation in Table A.4.

V. Discussion

Summary

The purpose for conducting these experiments was to maximize algal growth at the bench-scale by changing or enhancing different environmental conditions. Additionally, through its growth under these conditions, the alga was examined for its ability to sequester carbon dioxide. Using the resulting data, growth rates and yield values were calculated and compared to determine which conditions best supported growth. Following each experiment, the data were transferred to the other members of the research group for their use with the purpose of optimizing algal growth in the 3800 L photo-bioreactors (PBR).

Answers to Specific Research Questions

1. How is the alga affected by the use of different water sources? The PBRs maintained by the University of Dayton can run on tap water. It is essential to determine if the free chlorine or nutrients/contaminants within the tap water plumbed to the University of Dayton have a detrimental affect or a non-effect on mass algal cultivation within the PBRs.

The results of this experiment quickly demonstrated the nutrient dependent (exponential growth), nutrient independent (stationary growth/saturation), and nutrient inhibited (accelerated death phase) portions of *C. vulgaris'* life in a particular water type. In fact, it was demonstrated that all water types supported algal growth. But, some types supported growth better than others. De-ionized (D.I.) water provided the most rapid growth rate and highest yield of all water types; however, as has already been mentioned, the use of D.I. water as a source for the 3800 L photo-bioreactor (PBR) is not logistically

feasible. Therefore, it is appropriate to determine a suitable alternative. In this case, and as evidenced throughout the experimental time period, Charcoal Filtered Tap Water was sufficient to support algal growth at rates and yields consistent with the literature (Illman et al., 2000; Scragg et al., 2002; Hsieh and Wu, 2009; C. Yoo et al., 2010). Whether or not the water and media solutions were autoclaved did not seem to affect culture growth in a consistent manner. While growth was retarded significantly during Experiment 1 in the autoclaved culture, it was significantly enhanced during Experiment 2. Therefore, through this understanding of growth potential in various water configurations, the optimal water source for *C. vulgaris* growth appears to be Charcoal Filtered Tap Water as autoclaving is not feasible on the scale required for the 3800 L PBRs. This water source was recommended for use in mass algal culture and was used in subsequent experiments. Whole house charcoal filters are easy to install and inexpensive considering the scale of work being accomplished.

In many cases, the use of a Charcoal Filter may not be necessary; however, they are effective at filtering out hydrocarbons (including Volatile Organic Carbon), some larger bacteria (depending on the size of the filter membrane), and some free chlorine. Their continued use as a protective, albeit sometimes unnecessary, mechanism is recommended due to the fact that contaminant spikes may occur from time to time at levels that may adversely affect continuous growth in the 3800 L PBRs.

As can be seen in the abridged City of Dayton Consumer Confidence report, Table J.2 of Appendix J, average contaminant levels did not exceed their respective maximum contaminant level values (MCLs) in 2008 and there were no measured bacteria detections. However, assuming that elevated levels of free chlorine may negatively affect

the algal cultures, free chlorine levels were measured using a Hach test kit (Hach Pocket Colorimeter II, S/N – 00025444). The free chlorine levels for each water type were as listed below:

D.I. Water:	0.00 mg/L
Tap Water:	0.70 mg/L
Tap Water w/ Charcoal Filter:	0.05 mg/L
Tap Water w/ Charcoal Filter & Autoclave:	0.04 mg/L

Each of these values was below the average level listed in the consumer confidence report (1.125 mg/L) and appeared to have no impact on the growth of the algal culture in any of the water types.

As mentioned in Section 4, it was observed during Experiment 1 and Experiment 2 that some portion of the algal cells exhibited an adherence to the bottom of each culture flask (all cultures in both experiments, exceptions were the D.I. water cultures). Upon closer inspection it was apparent that some of the cells had formed a floc suspension within each culture flask as well. The lower absorbances observed as a result of this cell adherence and flocculation greatly affected the outcome of each culture's growth and my recommendation for use of an appropriate water type. However, these occurrences are vital to a successful culture program. Flocculation can play both positive and negative roles during micro-algal mass culture. During cultivation, one wants to produce as much biomass as possible in the most rapid manner possible. Auto-flocculation of algal cells would inhibit such an outcome. Conversely, during harvesting, auto-flocculation or mechanistic flocculation (raising the pH, introducing a flocculating agent) would be welcomed as it would greatly enhance the harvest. Auto-flocculation is onset through

any one of several processes. The first process involves a specific growth phase. During the exponential growth phase, negative surface charges in the culture cells are high and difficult to neutralize; the algae remain dispersed. As growth slows down, the negative surface charges on the cells become weaker and they begin to clump and settle to the culture bottom (Becker, 1994). A similar method involves calcium salts. Free calcium phosphate precipitate has a positive surface charge and may be absorbed by the algae to neutralize their negative surface charges. These types of flocculation will occur when elevated levels of calcium, magnesium, and phosphate are present, such as would occur in hard water enhanced with algal growth medium. Additionally, elevated pH levels can induce auto-flocculation. This type of flocculation is typically associated with CO_2 assimilation in the culture through photosynthesis yielding algal / nutrient as a co-precipitate. As CO_2 is removed from aqueous solution by the algae through photosynthesis, pH tends upward unless buffered in some way. As pH approaches a region from 8.5 – 9, $CaCO_3$ may precipitate out, dragging algal cells with it. This type of process may be the cause of the poor performance of the culture growing in autoclaved media during Experiment 1. As the water was heated in the autoclave, less CO_2 was able to dissolve causing a retardation of the pH lowering capacity that bubbling CO_2 has in an airlift system. This was observed as a white precipitate on the flask bottom. However, this precipitate was not analyzed in any manner due to the non-availability of appropriate hardware. Heath et al. found that in hard water and media containing ~ 68 mg/L soluble calcium, $CaCO_3$ precipitates out in batch culture taking algal cells with it in a floc (Heath et al., 1995). Becker lists this value as between 100 – 160 mg/L soluble calcium (Becker, 1994). However, the values present within the cultures investigated during Experiments

1 and 2 were only 27 mg/L soluble calcium but ~ 150 mg/L as $CaCO_3$ (mostly contributed by the tap water supplied by the City of Dayton). Thus, I am not inclined to attribute the auto-flocculation to those elevated calcium levels as Heath et al. observed. One last contributing method involves the interaction of the algal cells with bacteria or any of the organic exudates within the culture medium.

Any one of the mechanisms listed above could have contributed to the auto-flocculation observed in Experiments 1 and 2. One of the cultures in each of the first two experiments was autoclaved, temporarily elevating the pH and potentially precipitating $CaCO_3$. However, upon completion of the autoclave and re-introduction of CO_2 through a CO_2-in-air bubble stream, the $CaCO_3$ should have re-dissolved as the pH lowered accordingly. Therefore, this is not presumed to be the cause of the auto-flocculation. Additionally, cultures were not grown axenically. Therefore, any of the bacteria present within the culture solutions could have contributed to or caused the auto-flocculation. However, the D.I. water culture exhibited no cell adherence or auto-flocculation. If bacteria or cell exudates were the cause, then auto-flocculation would have been observed in the D.I. culture as well. Finally, I return to the elevated Ca^{2+}, Mg^{2+}, NO^{3-}, and PO_4^{3-} concentrations as potential causes of the auto-flocculation as co-precipitates. Considering the elevated levels of nutrients, it is presumed that conditions existed within each culture to allow the nutrients to complex as calcium phosphates and be absorbed in the algal cells in an effort to neutralize the negative surface charge exhibited by the cells. As these complexes were absorbed into the cells, they would produce the co-precipitate discussed in the paragraph above. This is the process assumed to cause the precipitate observed during Experiments 1 and 2. However, I must note here that in subsequent

experiments, none of the cultures produced a precipitate (including those grown in Charcoal Filtered Tap Water).

2. How does algal exposure to increasing CO_2 concentrations affect their growth, as compared to those concentrations available in the ambient atmosphere (0.04 % v/v)? An appropriate algae species must be capable of growing under high CO_2 concentrations (~15%), similar to that found in power plant flue gas. Does extreme pH, when driven by high concentrations of CO_2, negatively affect algal productivity?

C. vulgaris performed well over a wide range of CO_2 concentrations, exhibiting growth in all cultures except for the 100% CO_2 culture. In fact, growth rates and yields increased with increasing CO_2 concentration up to the 15% CO_2-in-air culture. At concentrations above 15%, the alga experienced decreasing growth with increasing CO_2 concentrations, or an inverse relationship to CO_2 concentration, and was unable to grow at concentrations of 100%. Doubling times of the alga were typically once per day with best performance observed in the 15% culture, exhibiting a doubling time over its exponential growth period of ~ 24.5 hours. Doubling times for each culture showed an inverse relationship with its respective increase or decrease in growth rate. In other words, from the ambient air culture through the 15% CO_2 culture, doubling times decreased with increasing CO_2 concentration, whereas doubling times increased with each successive culture above 15%. While doubling times are a bit different, the numbers uncovered in this experiment are consistent with literature values. For instance, Hanagata et al. discovered the same correlation between increasing CO_2-in-air concentrations and growth rates. For cultures grown in 10, 20, 30, 40, 50, 60, and 80%

CO_2-in-air, they observed doubling times of 18, 18, 22, 22, 28, 41, and 144 hours respectively (Hanagata et al., 1992). Widjaja et al. observed results in line with the findings here in this experiment and with those of Hanagata et al., identifying the same correlation of increasing yield and growth rate with increasing CO_2 concentration (Widjaja et al., 2009). Similarly, Yoo et al. observed increasing growth in its *C. vulgaris* cultures up through 18% CO_2-in-air with high yields of biomass (Yoo et al., 2009). Thus, based on the results here and on those found within the literature cited here, it appears as if this particular alga is appropriate for use in any remediation scheme that contains CO_2 gas in concentrations up through 50%, with ideal growth and yield at concentrations between 4% and 15% CO_2-in-air. Therefore, from the perspective of CO_2 tolerance, this species of algae will be appropriate for coal-fired power plant and Fischer-Tropsch plant flue gas remediation.

The principal concern with elevated CO_2 concentrations involves culture viability in a low pH environment, where pH tolerance (as a culture attribute) is just as important of a qualifying parameter as CO_2 concentration is. This is based in simple inorganic chemistry where unused aqueous CO_2 exists in solution as weak carbonic acid according to the following equilibrium equations:

$$CO_2\ (g) \leftrightarrow CO_2\ (aq) \leftrightarrow HCO_3^- \leftrightarrow CO_3^{2-}$$

where CO_2 (aq) is synonymous with H_2CO_3. Upon inoculation, within one day the pH dropped to ~ 4.75 and 4.5 in the 50% and 100% CO_2-in-air cultures, respectively. To prevent culture death, the pH was adjusted with 1 Molar KOH to ~ 6.6. By Day 2, the solutions were able to buffer themselves and gave pH readings around 6.2 for each culture. While experiencing lower pHs, cultures with lower CO_2 concentration were not

adversely affected, indicating two things; one, *C. vulgaris* will survive and grow at pHs of greater than 5, and two, the *C. vulgaris* is able to buffer itself to approximately neutral conditions. This concept was investigated in great detail by Sorokin. In preparing this media, there exists an assumption that concentrations of certain nutrients are abundant enough to provide proper buffer throughout observations. With freshly inoculated algal cells during this experiment, this assumption may be sound, since the over-abundance of phosphates in solution initially provides adequate buffering. As the cells grow, nutrients (phosphates included) are consumed or overwhelmed (as is the case with the 50% and 100% CO_2 cultures), reducing the chemical buffering capacity of the nutrient solution. If no artificial buffer is introduced, the algal solution must then rely on the carbonate system to accommodate. However, considering the flow rates of CO_2 introduced to the culture in this experiment, there was an overabundance of CO_2 in all cases dampening the buffer capacity of a normal aqueous system. Fortunately, other substances such as organic acids contribute to the buffering capacity developed in the process of algal growth (Sorokin, 1971). As daughter cells build up in culture and are released during cell reproduction, other organic constituents are released effectively buffering the solution. This accounts for the maintenance of culture pH after Day 2 of the experiment.

3. Is there an appreciable difference in growth rate for algae grown using commercial fertilizers over those media specialized for algal mass culture?

The plant fertilizers investigated here proved to be useful media substitutes, and in many cases facilitated growth in cultures that out-performed those cultures grown on the specialized Bold's media. Not only was growth similar or greater in cultures grown

on the commercial fertilizers, but it was usually more easily prepared and more cost efficient. For instance, BBM costs approximately 55.00 USD for 1 Liter of solution according to the UTEX website (www.utex.org, 2009 dollar value). The first nutrient source compared to BBM, called Scott's Cal-Mag (15-5-15) Peters® Excel®, costs approximately 0.50 USD for 1 Liter of solution (55 lb bag fertilizer, using 1 g/L concentration). The nutrient solution created using Bold's recipe referred to in Section 4 as "commercial fertilizer" was an amalgamation of several different types of fertilizers created to mimic BBM as best as possible (refer to Appendix J for the amounts and concentrations of each nutrient source). Therefore, its cost is slightly higher than the all-in-one Cal-Mag, but still more economical than analytical grade BBM nutrients. In a pilot study using a medium sized bioreactor (200 L), Peters® Excel® provided sufficient nutrients to support exponential growth in a *C. vulgaris* culture for 3-4 days (data not contained in this document). However, at day 5 the culture entered the stationary phase of growth and quickly proceeded to the accelerated death phase. The purpose for evaluating these alternate nutrient sources was three-fold; first, determine the suitability of such nutrients as a source that facilitates continuous mass culture of *C. vulgaris*, second, find an economically viable alternative to BBM, and third, find a media solution that can be prepared in an easy manner.

As reported in Section 4, Cal-Mag supported growth of *C. vulgaris*, but in questionable ways. In the solution of 1 g/L Cal-Mag, growth occurred immediately but seemed to peak at 0.275 g/L with A550s of ~ 1.300. Solutions of 2 g/L and 5 g/L exhibited maximum cell concentrations of 0.487 and 0.668 g/L, respectively, indicating that with greater concentration comes greater cell growth. However, in addition to the

lag phase discussed previously in Section 4 under the Experiment 2 heading, the 2 g/L and 5 g/L cultures exhibited very low growth rates, outside of the confidence interval expected for the control culture (0.504 : 0.642 day^{-1}). These results are helpful in the selection of an alternative nutrient source. With the ease of use and cost efficiency of Cal-Mag, one will sacrifice either yield of biomass, or time in waiting through the long lag-phase, in comparison to the control. Obviously, in continuous culture, neither of these outcomes would be desirable. Therefore, it is recommended that Cal-Mag Peters® Excel® 15-5-15 not be used as a sole nutrient source for *C. vulgaris* growth in the 3800 L PBRs.

The commercial fertilizer made up as BBM with 4 x $NaNO_3$ supported algal growth in all cases over Experiments 4 and 5, exceeding control cultures on both occasions. The nutrient media was prepared using several different fertilizer bags (purchased from local plant nursery). For this reason, the nutrient solution is a little more expensive and difficult to prepare compared to the Cal-Mag because it requires measured contributions from each source. But, it is only as difficult to prepare as the analytical grade BBM which also requires measured contributions from each analytical grade nutrient. Additionally, biomass yields of 2.322 g/L were achieved in ten days of growth during Experiment 4 (compared to lower Cal-Mag culture yields) when culture growth was halted. Growth during Experiment 5 was greater for each Commercial Fertilizer culture compared to all Cal-Mag cultures as well, which is striking considering growth overall during Experiment 5 appeared to be stunted (control culture yielded 0.857 g/L biomass and only a 0.461 day^{-1} growth rate).

EDTA was not included in one of the Commercial Fertilizer cultures during Experiment 4 to determine the contribution it makes toward culture viability in the prevention of potential trace metal toxicity. EDTA has been used in algal media for a number of years as a chelating agent to bind up metal ions in solution, particularly iron. As bound ions, they remain in solution but are less active when complexed with EDTA, only becoming active as the equilibrium within the media changes, releasing the ions back into solution to be used by the algal cells. Extensive research has been conducted regarding the effects of EDTA in mass algal culture. One study of note regarded *Chlorella* species growth as significantly affected by both the iron concentrations available in solution and the ratio of iron available to EDTA. They observed that both growth rate and final cell concentration in the medium were greater when supplemented with EDTA (Sung et al., 1998). During Experiment 4, *C. vulgaris* growth in media with EDTA out-performed the alga grown in media without EDTA; although, differences in growth were not statistically significant. The data indicate one of three outcomes regarding EDTA; 1) EDTA enhances algal growth by binding up free metal ions making them available over longer periods of time, 2) metal ion concentrations were not present at high enough levels to induce toxicity, or 3) EDTA does not enhance algal growth in the presence of metal ions provided at the indicated concentration levels. In any configuration, EDTA does not appear to inhibit algal growth itself. Therefore, it is recommended that it be included (as Bold's recipe indicates) in any commercial fertilizer recipe created (if not already present) to ensure trace metal concentrations do not exert a toxic affect on the algal culture. Additionally, it appears that Commercial Fertilizer prepared according to Bold's recipe with 4 x $NaNO_3$ is sufficient to maximize biomass

yield at optimal growth rates, as compared to performance of the control cultures in all other experiments. Therefore, it is recommended that the Commercial Fertilizer described above with EDTA be used as the media solution for the 3800 L PBRs and for mass algal culture.

4. Does photoperiod play an important role in algal growth? Is there a benefit to the algae associated with exposure to light at shorter time periods per day versus continuous exposure?

Photosynthesis is a process by which light energy is converted into chemical energy to be used within the cells. It then stands to reason that greater amounts of light energy lead to greater amounts of chemical energy within the cell (stored as starches), and thus greater biomass yields over time. Within the PBR, algal cells may become mutually shaded by other cells as they grow. Depending on the mixing rate within the PBR, the algal cells may enter shaded areas that emulate zero-irradiance or zero-light energy conditions. Additionally, if the PBRs are used outdoors the algae will undergo several hours of continuous darkness each day depending on the time of year. Therefore, it is important to identify growth rates and yield potentials for this particular alga under different periods of light and darkness that will indicate the type of growth rate or yield to be expected at higher algal cell concentrations. Additionally, knowledge of growth or lack thereof under varying periods of darkness will help us identify photo-period minimums that must be maintained within the larger 3800 L PBRs.

The effects of light cycles have been reported by other researchers (Janssen et al., 1999). According to Janssen et al., light was a limiting substrate within the PBR as cell

concentrations became high. As shading occurred at elevated concentrations, light intensity became another limiting factor where intensity was vastly different across the breadth of the PBR; however, the effects of this variation were not investigated in this experiment.

Photoperiod had a significant effect ($P < 0.0001$) on final cell density at the end of the 10-day experiment. Accordingly, the division rates of the cultures for each photoperiod were dependent upon the photoperiod. In the case of this experiment, an increase in photoperiod generated increases in growth rate and biomass yield, indicating that the irradiance level each culture was exposed to was not photo-saturating the cultures in an inhibitory manner.

In conducting this experiment, we strove to determine if similar growth rates and yields could be obtained in cultures exposed to fewer hours of light each day in an effort to maximize production while minimizing cost. The answer to that question is no, similar growth rates cannot be obtained using the same irradiance level but exposing each culture to shorter light:dark regimes. These findings are consistent across the literature and are indicative of an organism that derives its energy from a light source. For instance, Castenholz found that day length was directly associated with growth rate (Castenholz, 1964) and Paasche observed that growth was retarded in cultures receiving fewer than 16 hours of light per day (Paasche, 1967). Meseck et al. achieved similar results as they compared an alga's response to growth and nutrient uptake while varying photo-period and light intensity achieving growth rates of 0.61 day^{-1} in the 24:0 photo-period (light:dark) and 0.49 day^{-1} in the 16:8 photo-period (Meseck et al., 2005). These values compare nicely to the results here, which were 0.67 day^{-1} in the 24:0 photo-period and

0.55 day^{-1} in the 18:6 photo-period, for the latter a number slightly greater than Meseck et al. observed but still consistent with my conclusion that an increase in photo-period yields an increase in growth rate accordingly.

While growth was not as intense in the 18:6 photo-period cultures, it did occur at a rate of growth consistent with the confidence interval discussed in Section 4 of this document, with two of the cultures equaling (statistically) growth in the 24:0 photo-period culture. This may suggest that *C. vulgaris* is capable of storing sufficient energy to sustain cell growth over some short period of darkness. For instance, Jacob-Lopes et al. observed this in their 22:2 photo-period cultures as their growth was statistically equal to their 24:0 photo-period cultures (Jacob-Lopes et al., 2009). However, additional trials are required in the photo-period range of 22:2 and 20:4 to better ascertain the causes of this anomaly.

Based on the results observed here in this experiment, the alga requires continuous light to produce maximum biomass; however, some growth does occur during shorter photo-periods. The quantification of this potential is important as optimization of the PBR occurs. Additionally, as is the case with the University of Dayton Research Institute (UDRI) PBRs, hardware may limit the photo-period exposure configuration. But overall, when used indoors, the PBRs can easily be configured to operate at the required irradiance and photo-period to optimize growth. With time though and considering long-term operating costs, the PBRs could be transferred to an outdoor location where the sun would supply some of the required energy for photosynthesis. Knowledge of photo-period and irradiance limitations for this particular alga will allow us to quantify the amount of artificial light that is required for supplementation in any

greenhouse type of configuration in order to maximize growth. Currently, at UDRI this limitation does not appear to be a concern as the algae continuously flow through the tubular PBR where they are exposed to a photo-period of ~ 21.2:2.8 (light:dark), a photo-period for which the literature suggests supports growth rates that are statistically the same as a 24:0 photo-period (Jacob-Lopes et al., 2009). Therefore, it is recommended that researchers at UDRI continue to operate the PBRs at re-circulation flows of between 50% and 70% with irradiance levels of at least 40 μmol m^{-2} s^{-1} in order to ensure that the algal cells receive light throughout the day of a duration that maximizes growth. Refinement of re-circulation flow percentages is recommended for future research to determine if greater re-circulation enhances or diminishes growth. Recirculation is briefly discussed in section A.5 of Appendix A.

5. How is growth affected during scale-up through the introduction of algae at varying degrees of culture dilution?

Considering a 3800 L PBR, it was appropriate to determine how dilute of an algal culture we could use to directly scale-up to full PBR production in mass culture. The purpose of this was two-fold; one, given the shear volume of PBR, we did not want to overwhelm the relatively small volume and concentration of algal cells creating a toxic affect, whereby the nutrients in solution are present at great concentrations compared to the relatively small number of algal cells, and two, we wanted to minimize any lag phase stagnation that may be encountered through the introduction of these organisms into an environment whose conditions are very different from those in the previous setting. Scale-up had already occurred in a parallel process within the lab from 250 mL flasks to

2 L flasks and on to 20 L carboys. The process was simple and straightforward and was conducted under the exact same environmental conditions as these experiments took place. A550 readings of ~ 5 have been routinely achieved in the 20 L carboys throughout the course of these experiments. Accordingly, using Eq. 5.1, I was able to postulate an approximate A550 reading for inspection and possible use in future scale-up experiments:

$$C_1V_1 = C_2V_2 \quad \text{(Eq. 5.1)}$$

with my experimental values plugged in, the equation becomes:

$$(5)(20\ \text{L}) = X_2\ (3800\ \text{L})$$

$$X_2 = 0.0263$$

where *C* is Concentration (A550 in this case), *V* is the volume of culture, and X_2 is the absorbance of the algal solution after transfer to the 3800 L PBR. The purpose of investigating this application lies in the opportunity to maximize biomass production within the PBR without spending long periods of time in the scale-up process, moving from the 20 L carboy to a 100 or 200 L tank / cylinder and subsequently the PBR, which could take several weeks, as Anderson suggests (Anderson, 2005). It takes approximately 5 days to achieve an A550 reading of ~ 5 in a 20 L carboy after inoculating from the 250 mL flask, a 100-fold step-wise increase. Using the X_2 solution determined above, Experiment 3 was conducted to determine rates of growth in cultures at the micro-scale that mimics absorbance at the macro-scale. There was one limitation that requires qualification; this involved the micro-scale growth conducted in this experiment using an air-lift system where CO_2-in-air bubbles rise from the flask or cylinder bottom for culture aeration and mixing versus the 3800 L PBR tubular system

that relies on injected CO_2 dissolving into the medium and water flow for movement throughout the serpentine design and subsequent culture growth.

Using Equation 5.1, one can estimate the PBR's $A550_0$ upon incorporation of the 20 L carboy with A550 ~ 5. Experiment 3 was conducted to mimic this condition with the most dilute culture trio of flasks inoculated to an A550 ~ 0.025. Additional cultures were prepared in step-wise fashion at $A550_0$ ~ 0.050, 0.100, and 0.200 (control). The results of this experiment were promising in that significant time in scale-up can be saved through direct incorporation of at least 0.025 concentrated cultures. Growth rates and A550 for each culture are displayed below:

$A550_0$ ~0.200	Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	A550	0.195	0.439	0.912	1.491	1.850	4.008	4.373	5.352	5.589	6.116	6.358	6.640	7.089	7.571	8.626	9.464	10.604	11.242	13.202
	k (day^{-1})		0.812	0.730	0.492	0.216	0.773	0.087	0.202	0.043	0.090	0.039	0.043	0.065	0.066	0.130	0.093	0.114	0.058	0.161

$A550_0$ ~0.100	Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	A550	0.103	0.244	0.612	1.010	1.439	2.959	3.438	4.560	4.877	5.130	5.670	5.950	6.488	6.946	7.914	10.289	11.171	13.120	14.280
	k (day^{-1})		0.862	0.920	0.501	0.354	0.721	0.150	0.282	0.067	0.051	0.100	0.048	0.087	0.068	0.131	0.262	0.082	0.161	0.085

$A550_0$ ~0.050	Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	A550	0.053	0.125	0.318	0.736	1.287	2.658	3.248	4.421	4.726	5.287	5.613	6.082	6.681	7.352	7.819	10.282	12.589	14.373	16.882
	k (day^{-1})		0.849	0.937	0.838	0.559	0.725	0.200	0.308	0.067	0.112	0.060	0.080	0.094	0.096	0.062	0.274	0.202	0.133	0.161

$A550_0$ ~0.025	Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	A550	0.027	0.068	0.202	0.463	1.003	2.289	3.018	4.120	4.254	5.043	5.481	5.950	6.531	7.224	7.914	9.613	11.222	12.689	15.889
	k (day^{-1})		0.929	1.083	0.830	0.774	0.825	0.276	0.311	0.032	0.170	0.083	0.082	0.093	0.101	0.091	0.194	0.155	0.123	0.225

Table 5.1: Growth rates for Experiment 3 cultures.

As can be seen in each culture beginning at Day 1, all cultures inoculated were in exponential growth and experienced growth rates in excess of 0.693 day^{-1}. While cell concentration is greater in cultures inoculated at 0.200 and 0.100, growth rates were more intense in the dilute cultures. This can be explained by simple mass balance mechanics. Each culture was inoculated into a culture medium containing the same amount of

nutrients (Appendix J, Table J.1). Cultures with greater cell concentration, and thus more algal cells, consumed more of the nutrients in their respective flasks than the more dilute cultures did, even though each experienced similar growth rates. In other words, the nutrient demand made by the more dilute cultures was not as great initially as it was in the more concentrated cultures, leaving greater amounts of nutrients in solution for later in the growth period. The result of this, as depicted in Figure 4.8 and 4.9 and in Table 5.1 above, is a medium solution that supports growth for longer periods of time in the more dilute cultures. In fact, the more dilute cultures caught up with and surpassed the more concentrated cultures in the Day 8 – 11 timeframe, and subsequently surpassed them by Day 15. The experiment concluded on Day 18. At that point, the 0.050 culture had yielded the most biomass, but the 0.025 culture was exhibiting a higher rate of growth. It is expected, based on the growth that occurred throughout the experiment, that the 0.025 culture would have surpassed the growth of the 0.050 culture by Day 19 or Day 20.

The results of this experiment are quite intriguing. It suggests that an inoculum grown in a 20 L carboy to A550 ~ 5 could survive and thrive in a 3800 L PBR without additional scale-up. Some sources suggest a 1:10 scale-up ratio (Richmond, 2004; Anderson, 2005) yet here in this case, a 1:100 ratio appears to be adequate. However, these results are indicative of an air-lift system and may not directly apply to a tubular serpentine system. Additionally, the decision to skip an additional scale-up step and move directly to the 3800 L PBR with a dilute culture must be tempered by the decision to either build up biomass quickly or accumulate a greater yield of biomass over a longer period of time. The 0.025 culture took ten days to accumulate the same amount of

biomass accumulated in only seven days by the 0.200 culture (5.481 versus 5.352, respectively).

6. Do alternative forms of Nitrogen enhance or adversely affect the growth of *Chlorella vulgaris*, as compared to the standard Nitrogen type listed in Bold's recipe?

Growth rates and biomass yields of *C. vulgaris* were affected by the type of nitrogen used in the culture medium. This appears to be a result of multiple factors; the ones I focus on here are nitrogen uptake rates, biomass yield, and culture pH. The three main types of nitrogen investigated during Experiments 4 and 5 were nitrate, ammonia, and organic nitrogen (Urea in this case). Most algae can assimilate all three types of nitrogen and some cyanobacteria can even utilize atmospheric nitrogen (N_2) for growth under otherwise poor nitrogen conditions. But, *C. vulgaris* has been observed to assimilate the three main types of nitrogen (Yun et al., 1997; Illman et al., 2000; Converti et al., 2009; Hsieh and Wu, 2009). It has been suggested that ammonium or organic nitrogen, like Urea, is the preferred form of nitrogen because it requires less energy for the algae to assimilate into their cells; nitrate must be reduced to ammonium ion before it can be assimilated for growth (Becker, 1994). Becker continues in his writing to suggest that Urea is the best source of nitrogen for mass cultivation. Based on the results observed during Experiment 4 and 5, this seems to be the case. The alga cultivated in the medium with ACS Urea as its nitrogen type performed much better than other cultures in other nitrogen media. Even during Experiment 5, where pH was maintained around 6.6, the Prilled Commercial Urea cultures performed as well as the ACS grade nitrate control

culture, suggesting that Urea is the best source. In fact, from simple chemistry, as Urea is catalyzed by the algae, it results in a bicarbonate ion (or CO_2 aqueous) and two ammonia ions according to the following reaction:

$$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$$

where both ammonia and carbon dioxide are made available to the algae for use. From the chemistry of the reaction, it appears that Urea would make the best source, as it can be completely used by the algae without an additional exertion of energy. Referring to Figure 5.1, one can observe the enhanced growth in the ACS and Prilled Commercial Urea cultures:

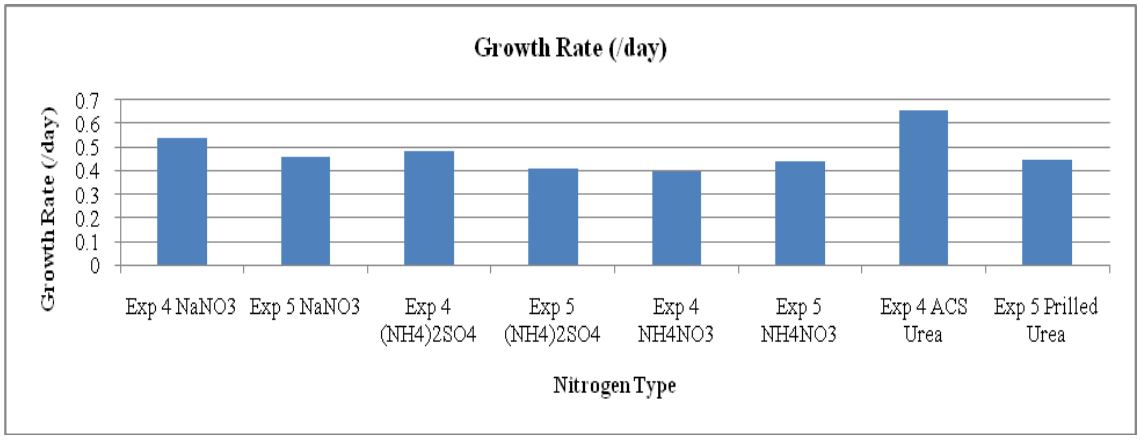


Figure 5.1: Comparison of growth rates for Experiments 4 & 5. Note the changes in the $(NH_4)_2SO_4$ culture when pH was maintained during Experiment 5.

However, an interesting observation occurred in the cultures grown with ammonium as their nitrogen type. Biomass accumulation over time was not necessarily linearly correlated with maximum growth rate. While more biomass was generated in the ACS Urea culture, some was sacrificed over the last three days of the experiment through respiration. In fact, this particular culture reached its biomass zenith on Day 7, and by Day 10 had sacrificed 18% of that peak (Day 10 final biomass concentration of 1.634

g/L). This indicates that the enhanced growth led to consumption of nutrients creating limiting conditions by Day 8. A depiction of maximum biomass accumulation (without regard to when the value was achieved) is available for reference below:

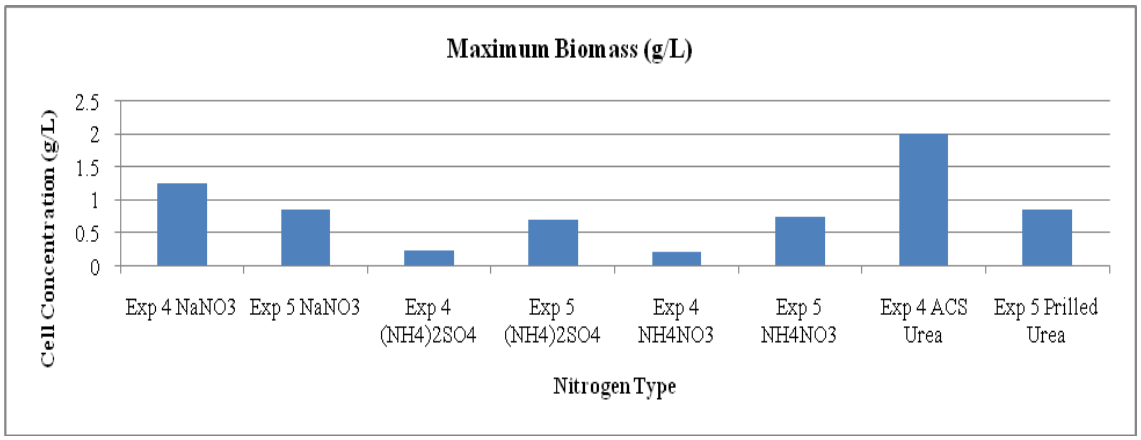


Figure 5.2: Maximum biomass achieved during Experiments 4 & 5.

Cultures grown on nitrate alone as the source continued to grow throughout the ten-day experiment (Experiment 4) indicating that nitrogen was being made available over time, through reduction, at a rate that was sufficient to allow the algae to grow at increased rates, but would not limit its growth.

The results from these two experiments indicated that *C. vulgaris* utilized ammonia (as Urea was broken down) in preference to nitrate as a nitrogen source, a result that is consistent with Yun et al., 1997. The results also indicated that the use of exotic types of nitrogen configurations, like ammonium sulfate and ammonium nitrate, could be detrimental to the health of the culture due to wide pH swings. As was presented in Section 4, with actively metabolizing cultures, culture pH dropped to the 3-4 pH range. Extended periods of time at this pH range effectively neutralized algal growth. As was evidenced in Experiment 5, although culture pH dropped to the 3-4 pH range in both the

ammonium sulfate and ammonium nitrate cultures, they were re-vitalized through periodic (daily) manual pH adjustment to 6.6. Apparently, if left alone to buffer, with two ammonium ions for every one sulfate ion the pH will decrease with ammonia uptake when bubbled with CO_2-enriched air. Due to the young age of the culture, algal metabolism is not active enough and thus not producing enough organic exudates to naturally buffer the solution (discussed in the preceding paragraphs under section 5, research question #2). The relatively small number of sulfate ions in culture cannot overcome the affect of ammonia uptake coupled with CO_2-enriched air. A similar set of circumstances may explain pH drop in the ammonium nitrate culture, although there was only one ammonium ion for every one nitrate ion.

Another interesting area that was left unexplained regards the preferential use of ammonical nitrogen over that available as nitrate. Becker suggests that nitrate is often not utilized by the algae until all ammonium is gone from culture medium, due to the metabolic energy requirements of its reduction (Becker, 1994). Therefore, when using both types of nitrogen in culture, one should observe a noticeable dip in culture growth followed by a rise in growth at presumably lower rates (based on findings presented in this thesis). No such dip was discovered in either Experiment 4 or 5. It is possible that a dip in growth may have occurred between Day 7 and Day 9 of Experiment 5 in the ammonium nitrate culture, but is impossible to say for sure based on the fact that only one data point was collected per day. Further research may be able to identify the cessation and resumption of growth with data points taken every hour.

From the findings presented here and in Section 4 regarding Experiment 4 and 5, it is clear that Urea was the optimal nitrogen type for *C. vulgaris* growth. Rapid growth

rate and high biomass yield indicate that this type of nitrogen has great potential for optimizing growth in mass culture over short periods of time. Use of ACS grade Urea or commercially available Urea must be further investigated as an adequate comparison could not be made between the two from Experiment 4 to Experiment 5. This suggestion is based on observations made regarding the overall reduced growth rates in all cultures observed during Experiment 5. Additionally, there appear to be no additional advantages gained when using both nitrate and ammonical types of nitrogen in culture. In fact, as observed here, its use may detract from culture productivity.

Conclusion

This thesis provided valuable information regarding the potential use of *C. vulgaris* for mass algal culture and sequestration of carbon dioxide. At the same time, it also provided some insight into the environmental parameters that most likely control growth in mass culture. Broad comparisons were made of many parameters throughout each experiment and provide a good basis for additional research toward the refinement of a process to maximize carbon dioxide sequestration and biomass production. This document can provide a framework for such work.

Limitations

Initially, the lack of research hardware and space (flasks, tubing, and tubing accessories) prevented a comparison of all Cal-Mag cultures in one experiment, forcing a qualitative and limited statistical comparison of cultures using additional controls. Additionally, due to available resources and time, a comparison of growth rates across an irradiance spectrum was not conducted. This would have afforded a visualization of the optimal light condition with which to expose each culture in order to maximize growth.

As was explained throughout the research, all culture growth occurred at ~ 40 μmol m^{-2} s^{-1}, a value that may not have maximized growth. Additional research in this area is warranted. Finally, although discussed in Section 2, due to the lack of available time and a workable solution, lipids productivity was not investigated during this work.

Opportunities for Further Research

1. Repeat this experiment with other algae species to determine their viability as both a good candidate for bio-fuels and for carbon dioxide sequestration.

2. Determine the plausibility of using wastewater from a local treatment plant as a quality substrate and water source for the particular alga. Wastewater contains many of the nutrients that the algae need to grow; however, they also contain many toxins. The proper characterization of a particular wastewater and the identification of an alga's ability to grow and bind up potential toxins in its biomass would be very beneficial.

3. Continue to investigate the green alga discussed here in this document. Two important parameters not optimized here were irradiance levels and lipids production for bio-fuels. The algae respond differently to increasing irradiance levels. However, the work presented in this thesis controlled the irradiance level at 40 μmol m^{-2} s^{-1}. Regarding lipids, algae produce different amounts in their biomass based on the type of substrate they grow on. In the past, research has focused on limiting the amount of Nitrogen in the substrate which leads to elevated lipid levels but deteriorating growth rates. Recent research though has determined that lipid levels can be maximized without sacrificing

growth rates. It may be appropriate to quantify these values for future use in the University of Dayton Research Institute's PBRs.

4. Examine *Chlorella vulgaris'* ability to grow on actual coal and Fischer-Tropsch power plant flue gas.

Appendix A: Standard Curve and Parameter Calculations

Dilution Rate	Volume of Algal Solution (mL)	Volume of Medium (mL)	Total Volume (mL)	Filter Weight (grams)	Filter Weight + Algae (grams)	Algal Mass (grams)	Cell Concentration (g/L)	Absorbance (550nm)	Absorbance Mean (550nm)
0x	0	50	50	0.02560	0.02560	0.00000	0.00000	0.00000	0.00000
0.1x	5	45	50	0.02571	0.02620	0.00049	0.0098000	0.118	0.118
								0.118	
								0.117	
0.2x	10	40	50	0.02455	0.02705	0.00250	0.0500000	0.233	0.233
								0.235	
								0.231	
0.3x	15	35	50	0.02600	0.02876	0.00276	0.0552000	0.329	0.332
								0.333	
								0.333	
0.5x	25	25	50	0.02558	0.03069	0.00511	0.1022000	0.558	0.559
								0.558	
								0.562	
0.7x	35	15	50	0.02494	0.03291	0.00797	0.1594000	0.753	0.753
								0.753	
								0.753	
1.0x	50	0	50	0.02561	0.03651	0.01090	0.2180000	1.014	1.015
								1.014	
								1.016	

Table A.1: Standard Curve Information, Experiment 1-3.

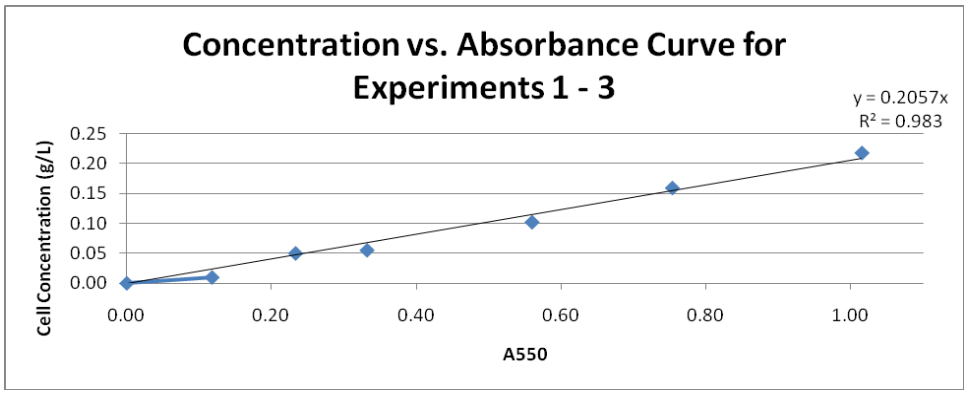


Figure A.1: Regression Equation for Standard Curve, Experiment 1-3.

Dilution Rate	Volume of Algal Solution (mL)	Volume of Medium (mL)	Total Volume (mL)	Filter Weight (grams)	Filter Weight + Algae (grams)	Algal Mass (grams)	Cell Concentration (g/L)	Absorbance (550nm)	Absorbance Mean (550nm)
0x	0	50	50	0.02560	0.02560	0.00000	0.00000	0.00000	0.00000
0.1x	5	45	50	0.02545	0.02566	0.00021	0.0042000	0.033	0.034
								0.033	
								0.035	
0.2x	10	40	50	0.02522	0.02567	0.00045	0.0090000	0.064	0.064
								0.065	
								0.064	
0.3x	15	35	50	0.02525	0.02606	0.00081	0.0162000	0.095	0.094
								0.093	
								0.095	
0.5x	25	25	50	0.02554	0.02674	0.00120	0.0240000	0.159	0.156
								0.157	
								0.152	
0.7x	35	15	50	0.02542	0.02723	0.00181	0.0362000	0.214	0.215
								0.218	
								0.213	
1.0x	50	0	50	0.02562	0.02792	0.00230	0.0460000	0.294	0.294
								0.294	
								0.295	

Table A.2: Standard Curve Information, Experiment 4-6.

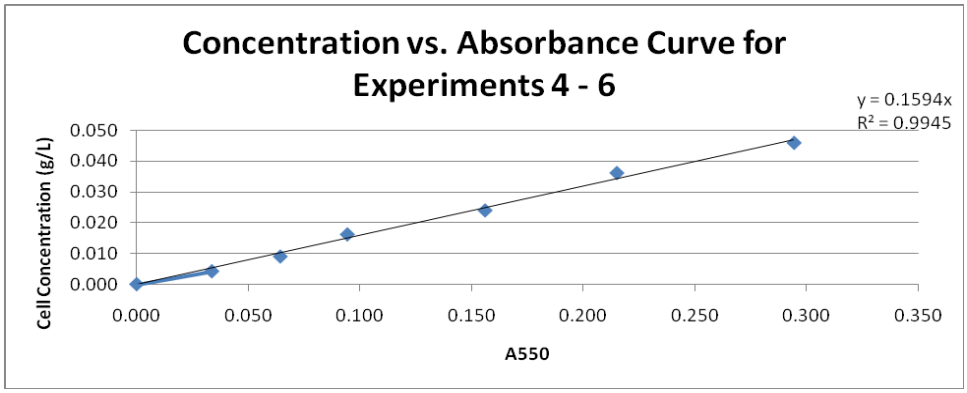


Figure A.2: Regression Equation for Standard Curve, Experiment 4-6.

Dilution Rate	Volume of Algal Solution (mL)	Volume of Medium (mL)	Total Volume (mL)	Filter Weight (grams)	Filter Weight + Algae (grams)	Algal Mass (grams)	Cell Concentration (g/L)	Absorbance (550nm)	Absorbance Mean (550nm)
0x	0	50	50	0.02560	0.02560	0.00000	0.00000	0.00000	0.00000
0.1x	5	45	50	0.02540	0.02569	0.00029	0.0058000	0.037	0.037
								0.038	
								0.037	
0.2x	10	40	50	0.02627	0.02678	0.00051	0.0102000	0.073	0.073
								0.073	
								0.073	
0.3x	15	35	50	0.02570	0.02665	0.00095	0.0190000	0.111	0.111
								0.111	
								0.111	
0.5x	25	25	50	0.02547	0.02680	0.00133	0.0266000	0.178	0.178
								0.178	
								0.179	
0.7x	35	15	50	0.02473	0.02679	0.00206	0.0412000	0.242	0.244
								0.244	
								0.245	
1.0x	50	0	50	0.02545	0.02822	0.00277	0.0554000	0.345	0.345
								0.345	
								0.344	

Table A.3: Standard Curve Information, Experiment 7.

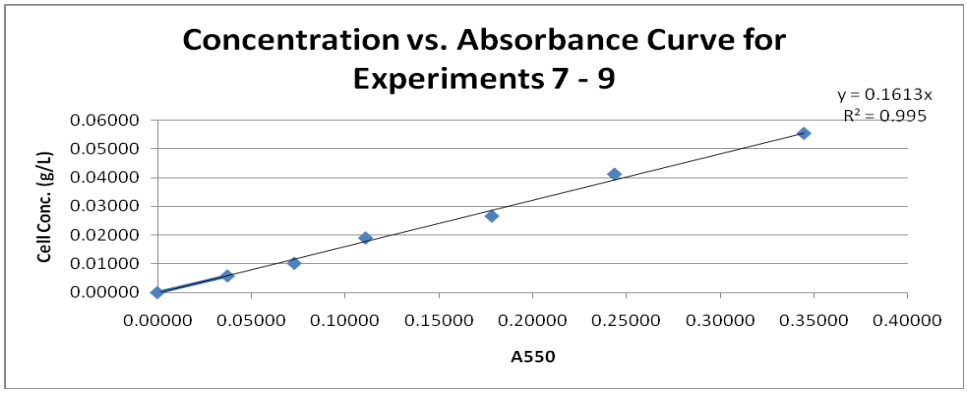


Figure A.3: Regression Equation for Standard Curve, Experiment 7.

Equation:		$A550_o$:	0.200				
y=0.1594x		$A550_{final}$:	2.000				
C_o:	**0.03188**						
C_{final}:	**0.3188**						
CO_2 : Biomass ratio of 1.83:1							
0.05834	**g CO_2/L**	CO_2 Sequestered at A550 reading (A550 - 0.2)					
0.583404	**g CO_2/L**	CO_2 Sequestered at A550 reading (A550 - 2.0)					
3800 L Photo-Bioreactor							
3800 L (0.12752 g CO_2/L) =			**221.694**	**g CO_2**			
3800 L (1.2752 g CO_2/L) =			**2216.935**	**g CO_2**			
Convert Grams of CO_2 into Moles and Liters of CO_2							
484.576 g CO_2 (1 mol/44 g CO_2) =				**5.038489**	**moles CO_2**		
4845.760 g CO_2 (1 mol/44 g CO_2) =				**50.38489**	**moles CO_2**		
V = nRT/P (Ideal Gas Law)							
V = (5.038489 moles)(.082 L atm/mol K)(298 K) / 1 atm =						**123.1205**	**L CO_2**
V = (50.38489 moles)(.082 L atm/mol K)(298 K) / 1 atm =						**1231.205**	**L CO_2**
Over the course of one day							
1 day (24 hrs/1 day)*(600 min/1 hour) = 1 day/1440 min							
*Take total volume of CO_2 required per day and divide by number of minutes per day							
85.50036	mL CO_2/min						
855.0036	mL CO_2/min						
To determine air flow rate that will provide a 4% CO2-in-air mixture of that rate above:							
Use equation: (CO_2 flow rate) = 0.04(x) and solve for x							
2137.51	***mL Air/min***	$A550_o$ - 0.200					
21375.1	***mL Air/min***	$A550_{final}$ - 2.00					

Table A.4: Theoretical CO_2 Calculation for Experiments 1-7 using one of the regression equations for example purposes.

Flow was measured at various re-circulation rates to determine the residence time of the algal solution in the tubes (exposed to light) and in the aeration tank (exposed to darkness). Flow was measured at various re-circulation rates for each PBR. Flow rates for each PBR are depicted in the graphs and tables below:

PBR 1	
Re-circulating Flow (%)	Volumetric Flow Rate (L/s)
50	1.00
60	1.66
70	2.35

PBR 2	
Re-circulating Flow (%)	Volumetric Flow Rate (L/s)
30	0.30
40	1.15
50	2.00

Table A.5 & A.6: Parameters for Photoperiod Calculation.

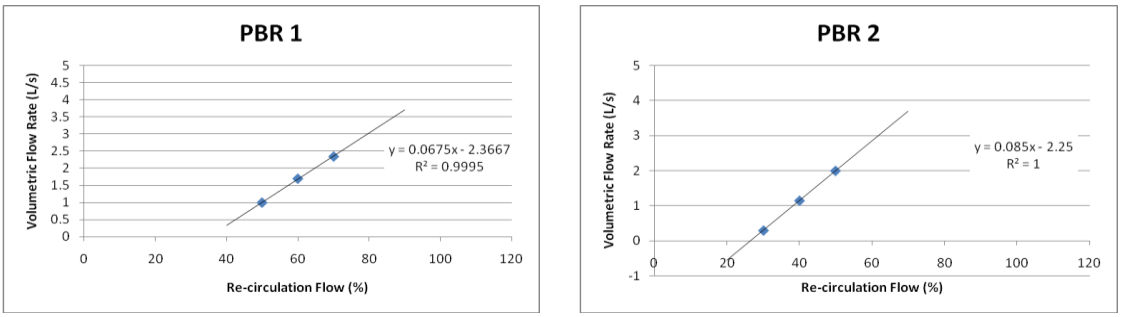


Figure A.4 & A.5: Re-circulation Graphs using Table A.5 & A.6 values above.

Therefore, with confidence, one can determine the flow rate for the algal solution in the 3800 L PBRs at any re-circulation rate. Typical re-circulation percentages range from 50 – 60%. Given water volume within the tank of 400 L and water volume within the tubes at 3000 L, residence time (hours) within each location can be determined easily. Dividing volume by flow rate at a particular percentage yields time (volume divided by volume/time yields time). For instance, at **50%** re-circulation flow for PBR 1:

Flow Rate = 1.0 L/s
Tank: 400 L / 1.0 L/s = 400 s (1 hour / 3600 s) = 0.11 hours
Tubes: 3000 L / 1.0 L/s = 3000 s (1 hour / 3600 s) = 0.83 hours
Addition of both areas equals 0.94 hours per complete cycle (one run through the entire PBR).

24 hours / 0.94 hours/cycle = 25.5 cycles per day
Tank: 25.5 cycles * 0.11 hours = 2.805 hours in the tank per day
Tubes: 25.5 cycles * 0.83 hours = 21.165 hours in the tubes per day

Similar calculations can be made for other re-circulation flows, but residence time within each area of the PBR per day does not dramatically change, resulting in an average photo-period within the UDRI PBRs of 21.2:2.8. Thus, adjustment of re-circulation flow does not alter photo-period. If photo-period adjustment is desired, tank volume must be adjusted.

Appendix B: Experimental Conditions

Species	Photoperiod	Irradiance(µmol m^{-2} s^{-1})	CO_2 Conc. (% in air)	Temp (°C)	Water Source
C. vulgaris	24:0	40	4	25	City of Dayton Tap Water
					City of Dayton Tap Water w/ Charcoal Filter & Autoclave
					City of Dayton Tap Water w/ Charcoal Filter
					D.I. Water

Table B.1: Water Source Experimental Conditions.

Species	Photoperiod	Irradiance(µmol m^{-2} s^{-1})	CO_2 Conc. (% in air)	Temp (°C)	Water Source
C. vulgaris	24:0	40	4	25	D.I. Water in BBM w/ 4 x $NaNO_3$
					City of Dayton Tap Water w/ Charcoal Filter in BBM w/ 4 x $NaNO_3$
					City of Dayton Tap Water w/ Charcoal Filter & Autoclaved in BBM w/ 4 x $NaNO_3$
					City of Dayton Tap Water w/ Charcoal Filter and 1 g/L Cal-Mag, 15-5-15
					City of Dayton Tap Water w/ Charcoal Filter and 2 g/L Cal-Mag, 15-5-15
					City of Dayton Tap Water w/ Charcoal Filter and 5 g/L Cal-Mag, 15-5-15

Table B.2: Alternate Nutrient Analysis Information.

Species	Volume of Algae Solution (mL) into 100 mL of Medium Solution	Initial A550	Initial Cell Concentration (g/L)	Water Source
C. vulgaris	2.00*	0.200	0.041	D.I. Water in BBM w/ 4 x $NaNO_3$
	1.00*	0.100	0.021	D.I. Water in BBM w/ 4 x $NaNO_3$
	0.50*	0.050	0.010	D.I. Water in BBM w/ 4 x $NaNO_3$
	0.25*	0.025	0.005	D.I. Water in BBM w/ 4 x $NaNO_3$

*From 50 mL aliquot centrifuge, placed in 100 mL of medium

Table B.3: Algal Dilution & Scale-Up Information.

Species	Photoperiod	Irradiance ($\mu mol\ m^{-2}\ s^{-1}$)	CO_2 Conc. (% in air)	Temp (°C)	Nitrogen Source	Nitrogen Source Concentration (mol/L)
C. vulgaris	24:0	40	4	25	$NaNO_3$	0.0117
					$[NH_4]_2SO_4$	0.00585
					NH_4NO_3	0.00585
					Urea (ACS)	0.00585
					Comm. Fertilizer (KNO_3) w/ EDTA	0.0117
					Comm. Fertilizer w/ EDTA & Prilled Urea	0.0117
					Comm. Fertilizer (KNO_3) w/ EDTA & Autoclave	0.00585
					Comm. Fertilizer (KNO_3) w/out EDTA	0.0117

Table B.4: Alternate Nitrogen Sources Experiment Information.

Species	Photoperiod	Irradiance($\mu mol\ m^{-2}\ s^{-1}$)	CO_2 Conc. (% in air)	Temp (°C)	Medium
C. vulgaris	24:0	40	4	25	BBM w/ 4 x $NaNO_3$
	18:6				
	12:12				
	6:18				

Table B.5: Photoperiod Information.

Species	Photoperiod	Irradiance($\mu mol\ m^{-2}\ s^{-1}$)	CO_2 Conc. (% in air)	Air Flow Rate (mL/min)	CO_2 Flow Rate (mL/min)	Temp (°C)	Medium
C. vulgaris	24:0	40	Ambient	1320	386 ppm	25	BBM w/ 4 x $NaNO_3$
			4	1500	60		
			10	1800	200		
			15	1700	300		
			20	1600	400		
			25	1125	375		
			30	1050	450		
			35	975	525		
			50	650	650		
			100	0	1000		

Table B.6: CO_2 Concentration Variables

Appendix C: Experiment 1 Data

Table C.1: Water Source Experiment, D.I. Water Data.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean (g/L)	Date
De-ionized Water	0	A1	0.266	0.267	0.055	0.055	14-Jul-09
			0.264		0.054		
			0.270		0.056		
		A2	0.270	0.266	0.056	0.055	
			0.263		0.054		
			0.264		0.054		
		A3	0.240	0.238	0.049	0.049	
			0.238		0.049		
			0.237		0.049		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	1	A1	0.273	0.274	0.056	0.056	15-Jul-09
			0.273		0.056		
			0.277		0.057		
		A2	0.250	0.252	0.051	0.052	
			0.254		0.052		
			0.252		0.052		
		A3	0.232	0.236	0.048	0.049	
			0.234		0.048		
			0.242		0.050		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	2	A1	0.426	0.430	0.088	0.089	16-Jul-09
			0.435		0.089		
			0.430		0.088		
		A2	0.387	0.387	0.080	0.080	
			0.388		0.080		
			0.385		0.079		
		A3	0.410	0.408	0.084	0.084	
			0.409		0.084		
			0.406		0.084		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	3	A1	1.069	1.064	0.220	0.219	17-Jul-09
			1.064		0.219		
			1.060		0.218		
		A2	1.011	1.045	0.208	0.215	
			1.062		0.218		
			1.062		0.218		

		A3	1.021	1.021	0.210	0.210	
			1.023		0.210		
			1.020		0.210		
De-ionized Water	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	A1	1.453	1.454	0.299	0.299	18-Jul-09
			1.458		0.300		
			1.450		0.298		
		A2	1.485	1.488	0.305	0.306	
			1.489		0.306		
			1.490		0.306		
		A3	1.448	1.453	0.298	0.299	
			1.455		0.299		
			1.457		0.300		
De-ionized Water	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	5	A1	1.686	1.679	0.347	0.345	19-Jul-09
			1.670		0.344		
			1.680		0.346		
		A2	1.610	1.609	0.331	0.331	
			1.609		0.331		
			1.608		0.331		
		A3	1.541	1.548	0.317	0.318	
			1.550		0.319		
			1.552		0.319		
De-ionized Water	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	A1	1.752	1.764	0.360	0.363	20-Jul-09
			1.768		0.364		
			1.772		0.365		
		A2	1.602	1.597	0.330	0.329	
			1.602		0.330		
			1.588		0.327		
		A3	1.583	1.583	0.326	0.326	
			1.583		0.326		
			1.583		0.326		

Table C.2: Water Source Experiment, Tap Water w/ Charcoal Filter & Autoclave Data.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & Autoclave	0	B1	0.255	0.254	0.052	0.052	14-Jul-09
			0.253		0.052		
			0.253		0.052		

		B2	0.261	0.263	0.054	0.054	
			0.264		0.054		
			0.265		0.055		
		B3	0.284	0.286	0.058	0.059	
			0.285		0.059		
			0.288		0.059		
Tap Water w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	1	B1	0.244	0.246	0.050	0.051	15-Jul-09
			0.249		0.051		
			0.244		0.050		
		B2	0.240	0.236	0.049	0.048	
			0.234		0.048		
			0.233		0.048		
		B3	0.256	0.256	0.053	0.053	
			0.250		0.051		
			0.262		0.054		
Tap Water w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	2	B1	0.424	0.426	0.087	0.088	16-Jul-09
			0.423		0.087		
			0.430		0.088		
		B2	0.375	0.378	0.077	0.078	
			0.379		0.078		
			0.380		0.078		
		B3	0.395	0.396	0.081	0.081	
			0.392		0.081		
			0.400		0.082		
Tap Water w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	3	B1	0.835	0.843	0.172	0.173	17-Jul-09
			0.834		0.172		
			0.859		0.177		
		B2	0.739	0.729	0.152	0.150	
			0.721		0.148		
			0.727		0.150		
		B3	0.853	0.852	0.175	0.175	
			0.857		0.176		
			0.845		0.174		
Tap Water w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	B1	1.285	1.270	0.264	0.261	18-Jul-09
			1.258		0.259		
			1.267		0.261		
		B2	1.18	1.179	0.243	0.243	

			1.173		0.241		
			1.185		0.244		
		B3	1.34	1.345	0.276	0.277	
			1.344		0.276		
			1.352		0.278		
Tap Water w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	5	B1	1.323	1.311	0.272	0.270	19-Jul-09
			1.304		0.268		
			1.305		0.268		
		B2	1.225	1.221	0.252	0.251	
			1.227		0.252		
			1.21		0.249		
		B3	1.383	1.383	0.284	0.285	
			1.381		0.284		
			1.386		0.285		
Tap Water w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	B1	1.256	1.258	0.258	0.259	20-Jul-09
			1.261		0.259		
			1.256		0.258		
		B2	1.085	1.089	0.223	0.224	
			1.101		0.226		
			1.08		0.222		
		B3	1.323	1.315	0.272	0.270	
			1.294		0.266		
			1.327		0.273		

Table C.3: Water Source Experiment, Tap Water w/ Charcoal Filter Data.

TW w/ Charcoal Filter	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	0	C1	0.301	0.305	0.062	0.063	14-Jul-09
			0.305		0.063		
			0.308		0.063		
		C2	0.301	0.302	0.062	0.062	
			0.302		0.062		
			0.303		0.062		
		C3	0.264	0.266	0.054	0.055	
			0.270		0.056		
			0.264		0.054		
TW w/ Charcoal Filter	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	1	C1	0.257	0.259	0.053	0.053	15-Jul-09

			0.263		0.054		
			0.258		0.053		
		C2	0.263	0.257	0.054	0.053	
			0.252		0.052		
			0.256		0.053		
		C3	0.221	0.224	0.045	0.046	
			0.223		0.046		
			0.227		0.047		
TW w/ Charcoal Filter	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	2	C1	0.416	0.428	0.086	0.088	16-Jul-09
			0.433		0.089		
			0.435		0.089		
		C2	0.456	0.459	0.094	0.094	
			0.460		0.095		
			0.461		0.095		
		C3	0.478	0.482	0.098	0.099	
			0.480		0.099		
			0.487		0.100		
TW w/ Charcoal Filter	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	3	C1	1.097	1.109	0.226	0.228	17-Jul-09
			1.122		0.231		
			1.107		0.228		
		C2	1.070	1.084	0.220	0.223	
			1.080		0.222		
			1.102		0.227		
		C3	1.004	1.004	0.207	0.207	
			1.001		0.206		
			1.008		0.207		
TW w/ Charcoal Filter	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	C1	1.355	1.362	0.279	0.280	18-Jul-09
			1.365		0.281		
			1.366		0.281		
		C2	1.345	1.364	0.277	0.281	
			1.363		0.280		
			1.383		0.284		
		C3	1.250	1.273	0.257	0.262	
			1.258		0.259		
			1.310		0.269		
TW w/ Charcoal Filter	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	5	C1	1.795	1.802	0.369	0.371	19-Jul-09
			1.802		0.371		
			1.809		0.372		

		C2	1.500	1.517	0.309	0.312	
			1.486		0.306		
			1.566		0.322		
		C3	1.372	1.350	0.282	0.278	
			1.378		0.283		
			1.300		0.267		
TW w/ Charcoal Filter	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	C1	1.885	1.890	0.388	0.389	20-Jul-09
			1.883		0.387		
			1.901		0.391		
		C2	1.532	1.492	0.315	0.307	
			1.476		0.304		
			1.467		0.302		
		C3	1.305	1.319	0.268	0.271	
			1.329		0.273		
			1.324		0.272		

Table C.4: Water Source Experiment, Tap Water Data.

Tap Water	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	0	D1	0.224	0.223	0.046	0.046	14-Jul-09
			0.223		0.046		
			0.223		0.046		
		D2	0.234	0.235	0.048	0.048	
			0.237		0.049		
			0.235		0.048		
		D3	0.253	0.254	0.052	0.052	
			0.256		0.053		
			0.253		0.052		
Tap Water	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	1	D1	0.201	0.204	0.041	0.042	15-Jul-09
			0.207		0.043		
			0.203		0.042		
		D2	0.223	0.226	0.046	0.047	
			0.229		0.047		
			0.227		0.047		
		D3	0.198	0.199	0.041	0.041	
			0.204		0.042		
			0.195		0.040		
Tap Water	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	2	D1	0.387	0.383	0.080	0.079	16-Jul-09

			0.388		0.080		
			0.375		0.077		
			0.459		0.094		
		D2	0.468	0.466	0.096	0.096	
			0.471		0.097		
			0.35		0.072		
		D3	0.355	0.361	0.073	0.074	
			0.379		0.078		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
			1.015		0.209		
		D1	1.032	1.022	0.212	0.210	
			1.02		0.210		
			1.098		0.226		
Tap Water	3	D2	1.095	1.096	0.225	0.225	17-Jul-09
			1.095		0.225		
			0.941		0.194		
		D3	0.974	0.967	0.200	0.199	
			0.987		0.203		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
			1.413		0.291		
		D1	1.459	1.429	0.300	0.294	
			1.415		0.291		
			1.345		0.277		
Tap Water	4	D2	1.348	1.336	0.277	0.275	18-Jul-09
			1.315		0.270		
			1.211		0.249		
		D3	1.215	1.215	0.250	0.250	
			1.218		0.251		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
			1.366		0.281		
		D1	1.37	1.370	0.282	0.282	
			1.373		0.282		
			1.456		0.299		
Tap Water	5	D2	1.435	1.443	0.295	0.297	19-Jul-09
			1.438		0.296		
			1.704		0.351		
		D3	1.701	1.706	0.350	0.351	
			1.712		0.352		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
			1.415		0.291		
Tap Water	6	D1	1.426	1.414	0.293	0.291	20-Jul-09
			1.4		0.288		

		D2	1.54	1.543	0.317	0.317	
			1.548		0.318		
			1.542		0.317		
		D3	1.827	1.847	0.376	0.380	
			1.855		0.382		
			1.858		0.382		

Appendix D: Experiment 2 Data

Table D.1: Alternate Nutrient Source Experiment, D.I. Water Data.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	0	A1	0.227	0.232	0.047	0.048	21-Jul-09
			0.235		0.048		
			0.234		0.048		
		A2	0.241	0.243	0.050	0.050	
			0.243		0.050		
			0.244		0.050		
		A3	0.224	0.224	0.046	0.046	
			0.221		0.045		
			0.226		0.046		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	1	A1	0.307	0.307	0.063	0.063	22-Jul-09
			0.305		0.063		
			0.308		0.063		
		A2	0.360	0.365	0.074	0.075	
			0.367		0.075		
			0.369		0.076		
		A3	0.293	0.294	0.060	0.060	
			0.294		0.060		
			0.295		0.061		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	2	A1	0.423	0.425	0.087	0.087	23-Jul-09
			0.428		0.088		
			0.424		0.087		
		A2	0.465	0.474	0.096	0.097	
			0.477		0.098		
			0.479		0.099		
		A3	0.428	0.429	0.088	0.088	
			0.434		0.089		
			0.426		0.088		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	3	A1	0.532	0.542	0.109	0.112	24-Jul-09
			0.545		0.112		
			0.550		0.113		
		A2	0.674	0.676	0.139	0.139	

			0.676		0.139		
			0.677		0.139		
		A3	0.832	0.828	0.171	0.170	
			0.824		0.169		
			0.827		0.170		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	4	A1	0.702	0.712	0.144	0.147	25-Jul-09
			0.729		0.150		
			0.706		0.145		
		A2	1.163	1.188	0.239	0.244	
			1.199		0.247		
			1.202		0.247		
		A3	1.272	1.268	0.262	0.261	
			1.269		0.261		
			1.263		0.260		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	5	A1	1.150	1.152	0.237	0.237	26-Jul-09
			1.131		0.233		
			1.176		0.242		
		A2	2.302	2.333	0.474	0.480	
			2.344		0.482		
			2.354		0.484		
		A3	2.400	2.408	0.494	0.495	
			2.394		0.492		
			2.430		0.500		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	6	A1	4.236	4.109	0.871	0.845	27-Jul-09
			4.040		0.831		
			4.052		0.833		
		A2	3.915	3.938	0.805	0.810	
			4.010		0.825		
			3.890		0.800		
		A3	3.980	3.932	0.819	0.809	
			3.910		0.804		
			3.905		0.803		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
De-ionized Water	7	A1	3.785	3.842	0.779	0.790	28-Jul-09
			3.805		0.783		
			3.935		0.809		
		A2	5.025	5.030	1.034	1.035	

			5.045		1.038		
			5.020		1.033		
		A3	5.415		1.114		
			5.485	5.445	1.128	1.120	
			5.435		1.118		
De-ionized Water	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	A1	4.325		0.890		
			4.360	4.387	0.897	0.902	
			4.475		0.921		
		A2	4.945		1.017		
			4.795	4.917	0.986	1.011	29-Jul-09
			5.010		1.031		
		A3	5.170		1.063		
			5.510	5.408	1.133	1.112	
			5.545		1.141		

Table D.2: Alternate Nutrient Source Experiment, Tap Water w/ Charcoal Filter Data.

Tap Water w/ CF	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	0	B1	0.245		0.050		
			0.245	0.246	0.050	0.051	
			0.249		0.051		
		B2	0.248		0.051		
			0.249	0.251	0.051	0.052	21-Jul-09
			0.255		0.052		
		B3	0.256		0.053		
			0.257	0.257	0.053	0.053	
			0.257		0.053		
Tap Water w/ CF	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	1	B1	0.43		0.088		
			0.43	0.429	0.088	0.088	
			0.428		0.088		
		B2	0.429		0.088		
			0.436	0.433	0.090	0.089	22-Jul-09
			0.435		0.089		
		B3	0.362		0.074		
			0.373	0.370	0.077	0.076	
			0.376		0.077		
Tap Water w/ CF	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	2	B1	0.63		0.130		
			0.633	0.633	0.130	0.130	23-Jul-09

			0.635		0.131		
		B2	0.64	0.636	0.132	0.131	
			0.634		0.130		
			0.633		0.130		
		B3	0.305	0.309	0.063	0.064	
			0.309		0.064		
			0.314		0.065		
Tap Water w/ CF	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	3	B1	0.997	0.997	0.205	0.205	24-Jul-09
			0.997		0.205		
			0.996		0.205		
		B2	0.982	0.984	0.202	0.202	
			0.984		0.202		
			0.987		0.203		
		B3	0.347	0.348	0.071	0.072	
			0.35		0.072		
			0.348		0.072		
Tap Water w/ CF	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	4	B1	1.223	1.208	0.252	0.249	25-Jul-09
			1.206		0.248		
			1.196		0.246		
		B2	1.333	1.329	0.274	0.273	
			1.333		0.274		
			1.322		0.272		
		B3	0.422	0.427	0.087	0.088	
			0.413		0.085		
			0.446		0.092		
Tap Water w/ CF	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	5	B1	1.56	1.453	0.321	0.299	26-Jul-09
			1.402		0.288		
			1.396		0.287		
		B2	2.152	2.133	0.443	0.439	
			2.172		0.447		
			2.076		0.427		
		B3	1.308	1.317	0.269	0.271	
			1.342		0.276		
			1.3		0.267		
Tap Water w/ CF	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	6	B1	1.915	1.887	0.394	0.388	27-Jul-09
			1.865		0.384		
			1.88		0.387		
		B2	2.285	2.275	0.470	0.468	

			2.195		0.452		
			2.345		0.482		
		B3	1.18		0.243		
			1.095	1.138	0.225	0.234	
			1.14		0.234		
Tap Water w/ CF	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	7	B1	2.55		0.525		
			3.335	2.855	0.686	0.587	
			2.68		0.551		
		B2	3.85		0.792		
			3.78	3.812	0.778	0.784	28-Jul-09
			3.805		0.783		
		B3	1.86		0.383		
			1.85	1.862	0.381	0.383	
			1.875		0.386		
Tap Water w/ CF	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	8	B1	2.81		0.578		
			2.54	2.643	0.522	0.544	
			2.58		0.531		
		B2	2.975		0.612		
			3.025	3.015	0.622	0.620	29-Jul-09
			3.045		0.626		
		B3	1.245		0.256		
			1.175	1.223	0.242	0.252	
			1.25		0.257		

Table D.3: Alternate Nutrient Source Experiment, Tap Water w/ Charcoal Filter & Autoclave Data.

TW w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	0	C1	0.241		0.050		
			0.240	0.240	0.049	0.049	
			0.240		0.049		
		C2	0.244		0.050		
			0.245	0.245	0.050	0.050	21-Jul-09
			0.246		0.051		
		C3	0.232		0.048		
			0.230	0.231	0.047	0.048	
			0.231		0.048		
TW w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	1	C1	0.409	0.404	0.084	0.083	22-Jul-09

			0.402		0.083		
			0.400		0.082		
		C2	0.442	0.444	0.091	0.091	
			0.447		0.092		
			0.444		0.091		
		C3	0.442	0.441	0.091	0.091	
			0.439		0.090		
			0.441		0.091		
TW w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	2	C1	0.630	0.634	0.130	0.130	23-Jul-09
			0.635		0.131		
			0.638		0.131		
		C2	0.791	0.796	0.163	0.164	
			0.799		0.164		
			0.799		0.164		
		C3	0.705	0.704	0.145	0.145	
			0.704		0.145		
			0.704		0.145		
TW w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	3	C1	0.827	0.826	0.170	0.170	24-Jul-09
			0.826		0.170		
			0.824		0.169		
		C2	1.089	1.092	0.224	0.225	
			1.092		0.225		
			1.096		0.225		
		C3	0.984	0.982	0.202	0.202	
			0.982		0.202		
			0.980		0.202		
TW w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	C1	1.040	1.083	0.214	0.223	25-Jul-09
			1.098		0.226		
			1.112		0.229		
		C2	1.356	1.375	0.279	0.283	
			1.392		0.286		
			1.376		0.283		
		C3	1.382	1.404	0.284	0.289	
			1.427		0.294		
			1.404		0.289		
TW w/ Charcoal Filter & Autoclave	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	5	C1	1.778	1.771	0.366	0.364	26-Jul-09
			1.762		0.362		
			1.774		0.365		

		C2	2.656	2.660	0.546	0.547	
			2.666		0.548		
			2.658		0.547		
		C3	2.126	2.125	0.437	0.437	
			2.108		0.434		
			2.140		0.440		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
TW w/ Charcoal Filter & Autoclave	6	C1	1.755	1.688	0.361	0.347	27-Jul-09
			1.600		0.329		
			1.710		0.352		
		C2	4.665	4.723	0.960	0.972	
			4.760		0.979		
			4.745		0.976		
		C3	3.195	3.093	0.657	0.636	
			3.045		0.626		
			3.040		0.625		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
TW w/ Charcoal Filter & Autoclave	7	C1	2.140	2.308	0.440	0.475	28-Jul-09
			2.375		0.489		
			2.410		0.496		
		C2	5.950	5.920	1.224	1.218	
			5.600		1.152		
			6.210		1.277		
		C3	4.000	4.018	0.823	0.827	
			3.990		0.821		
			4.065		0.836		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
TW w/ Charcoal Filter & Autoclave	8	C1	2.650	2.625	0.545	0.540	29-Jul-09
			2.550		0.525		
			2.675		0.550		
		C2	5.920	6.820	1.218	1.403	
			7.870		1.619		
			6.670		1.372		
		C3	2.675	2.647	0.550	0.544	
			2.625		0.540		
			2.640		0.543		

Table D.4: Data for Alternate Nutrient Source Experiment, Tap Water w/ Charcoal Filter & 1 g/L Scott's Peters® Excel® Cal-Mag.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 1 g/L Cal-Mag	0	D1	0.233	0.234	0.048	0.048	21-Jul-09
			0.235		0.048		
			0.233		0.048		
		D2	0.229	0.229	0.047	0.047	
			0.230		0.047		
			0.228		0.047		
		D3	0.239	0.239	0.049	0.049	
			0.239		0.049		
			0.239		0.049		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 1 g/L Cal-Mag	1	D1	0.245	0.237	0.050	0.049	22-Jul-09
			0.233		0.048		
			0.232		0.048		
		D2	0.167	0.164	0.034	0.034	
			0.157		0.032		
			0.168		0.035		
		D3	0.190	0.196	0.039	0.040	
			0.203		0.042		
			0.194		0.040		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 1 g/L Cal-Mag	2	D1	0.593	0.592	0.122	0.122	23-Jul-09
			0.588		0.121		
			0.595		0.122		
		D2	0.425	0.429	0.087	0.088	
			0.429		0.088		
			0.432		0.089		
		D3	0.363	0.363	0.075	0.075	
			0.361		0.074		
			0.366		0.075		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 1 g/L Cal-Mag	3	D1	0.744	0.750	0.153	0.154	24-Jul-09
			0.747		0.154		
			0.759		0.156		
		D2	0.832	0.837	0.171	0.172	
			0.837		0.172		
			0.842		0.173		
		D3	0.816	0.820	0.168	0.169	
			0.819		0.168		

			0.825		0.170		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 1 g/L Cal-Mag	4	D1	1.146	1.111	0.236	0.229	25-Jul-09
			1.104		0.227		
			1.084		0.223		
		D2	1.169	1.170	0.240	0.241	
			1.174		0.241		
			1.168		0.240		
		D3	1.129	1.121	0.232	0.231	
			1.119		0.230		
			1.116		0.230		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 1 g/L Cal-Mag	5	D1	1.285	1.296	0.264	0.267	26-Jul-09
			1.346		0.277		
			1.257		0.259		
		D2	1.274	1.276	0.262	0.262	
			1.283		0.264		
			1.271		0.261		
		D3	1.293	1.271	0.266	0.262	
			1.256		0.258		
			1.265		0.260		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 1 g/L Cal-Mag	6	D1	1.4	1.500	0.288	0.309	27-Jul-09
			1.6		0.329		
			1.5		0.309		
		D2	1.288	1.214	0.265	0.250	
			1.16		0.239		
			1.194		0.246		
		D3	1.132	1.302	0.233	0.268	
			1.59		0.327		
			1.184		0.244		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 1 g/L Cal-Mag	7	D1	1.61	1.493	0.331	0.307	28-Jul-09
			1.255		0.258		
			1.615		0.332		
		D2	1.39	1.397	0.286	0.287	
			1.425		0.293		
			1.375		0.283		
		D3	0.905	0.970	0.186	0.200	
			0.975		0.201		
			1.03		0.212		
Tap Water w/ Charcoal Filter &	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date

1 g/L Cal-Mag	8	D1	1.366	1.267	0.281	0.261	29-Jul-09
			1.21		0.249		
			1.226		0.252		
		D2	0.976	0.980	0.201	0.202	
			0.986		0.203		
			0.979		0.201		
		D3	0.914	0.867	0.188	0.178	
			0.853		0.175		
			0.833		0.171		

Table D.5: Data for Alternate Nutrient Source Experiment, Tap Water w/ Charcoal Filter & 2 g/L Scott's Peters® Excel® Cal-Mag.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	0	D1	0.201	0.201	0.032	0.032	9-Nov-09
			0.201		0.032		
			0.201		0.032		
		D2	0.197	0.197	0.032	0.032	
			0.197		0.032		
			0.197		0.032		
		D3	0.206	0.206	0.033	0.033	
			0.206		0.033		
			0.206		0.033		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	1	D1	0.230	0.231	0.037	0.037	10-Nov-09
			0.231		0.037		
			0.232		0.037		
		D2	0.189	0.187	0.030	0.030	
			0.187		0.030		
			0.186		0.030		
		D3	0.195	0.195	0.031	0.031	
			0.195		0.031		
			0.194		0.031		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	2	D1	0.226	0.225	0.036	0.036	11-Nov-09
			0.225		0.036		
			0.223		0.036		
		D2	0.163	0.163	0.026	0.026	
			0.163		0.026		
			0.163		0.026		
		D3	0.165	0.165	0.027	0.027	
			0.165		0.027		

			0.165		0.027		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	3	D1	0.203	0.204	0.033	0.033	12-Nov-09
			0.203		0.033		
			0.205		0.033		
		D2	0.123	0.122	0.020	0.020	
			0.120		0.019		
			0.122		0.020		
		D3	0.149	0.150	0.024	0.024	
			0.151		0.024		
			0.151		0.024		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	4	D1	0.179	0.181	0.029	0.029	13-Nov-09
			0.180		0.029		
			0.183		0.030		
		D2	0.101	0.102	0.016	0.017	
			0.103		0.017		
			0.103		0.017		
		D3	0.133	0.133	0.021	0.021	
			0.132		0.021		
			0.134		0.022		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	5	D1	0.257	0.256	0.041	0.041	14-Nov-09
			0.258		0.042		
			0.254		0.041		
		D2	0.139	0.137	0.022	0.022	
			0.134		0.022		
			0.137		0.022		
		D3	0.196	0.196	0.032	0.032	
			0.196		0.032		
			0.196		0.032		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	6	D1	0.376	0.374	0.061	0.060	15-Nov-09
			0.373		0.060		
			0.374		0.060		
		D2	0.152	0.154	0.025	0.025	
			0.156		0.025		
			0.153		0.025		
		D3	0.269	0.268	0.043	0.043	
			0.270		0.044		
			0.266		0.043		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	7	D1	0.531	0.531	0.086	0.086	16-Nov-09
			0.532		0.086		
			0.530		0.085		
		D2	0.171	0.172	0.028	0.028	
			0.173		0.028		
			0.173		0.028		
		D3	0.291	0.291	0.047	0.047	
			0.291		0.047		
			0.292		0.047		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	8	D1	0.851	0.852	0.137	0.137	17-Nov-09
			0.854		0.138		
			0.852		0.137		
		D2	0.313	0.312	0.050	0.050	
			0.313		0.050		
			0.311		0.050		
		D3	0.602	0.604	0.097	0.097	
			0.603		0.097		
			0.606		0.098		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	9	D1	0.560	0.567	0.090	0.091	18-Nov-09
			0.570		0.092		
			0.570		0.092		
		D2	0.464	0.464	0.075	0.075	
			0.465		0.075		
			0.464		0.075		
		D3	0.802	0.803	0.129	0.130	
			0.803		0.130		
			0.805		0.130		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	10	D1	0.903	0.904	0.146	0.146	19-Nov-09
			0.904		0.146		
			0.905		0.146		
		D2	0.560	0.553	0.090	0.089	
			0.560		0.090		
			0.540		0.087		
		D3	0.850	0.843	0.137	0.136	
			0.840		0.135		
			0.840		0.135		
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	11	D1	1.610	1.620	0.260	0.261	20-Nov-09

			1.620		0.261		
			1.630		0.263		
		D2	1.180		0.190		
			1.170	1.173	0.189	0.189	
			1.170		0.189		
		D3	1.590		0.256		
			1.580	1.587	0.255	0.256	
			1.590		0.256		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		D1	2.560		0.413		
			2.560	2.550	0.413	0.411	
			2.530		0.408		
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag		D2	2.080		0.336		
	12		2.090	2.077	0.337	0.335	21-Nov-09
			2.060		0.332		
		D3	2.530		0.408		
			2.540	2.533	0.410	0.409	
			2.530		0.408		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		D1	3.030		0.489		
			3.040	3.033	0.490	0.489	
			3.030		0.489		
Tap Water w/ Charcoal Filter & 2 g/L Cal-Mag		D2	3.250		0.524		
	13		3.240	3.250	0.523	0.524	22-Nov-09
			3.260		0.526		
		D3	2.770		0.447		
			2.780	2.780	0.448	0.448	
			2.790		0.450		

Table D.6: Data for Alternate Nutrient Source Experiment, Tap Water w/ Charcoal Filter & 5 g/L Scott's Peters® Excel® Cal-Mag.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		E1	0.214		0.035		
			0.214	0.214	0.035	0.035	
			0.214		0.035		
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag		E2	0.214		0.035		
	0		0.214	0.214	0.035	0.035	9-Nov-09
			0.214		0.035		
		E3	0.206		0.033		
			0.206	0.206	0.033	0.033	
			0.206		0.033		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	1	E1	0.280	0.278	0.045	0.045	10-Nov-09
			0.279		0.045		
			0.276		0.045		
		E2	0.248	0.248	0.040	0.040	
			0.248		0.040		
			0.248		0.040		
		E3	0.209	0.210	0.034	0.034	
			0.210		0.034		
			0.211		0.034		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	2	E1	0.345	0.345	0.056	0.056	11-Nov-09
			0.345		0.056		
			0.346		0.056		
		E2	0.245	0.245	0.040	0.040	
			0.244		0.039		
			0.246		0.040		
		E3	0.204	0.205	0.033	0.033	
			0.205		0.033		
			0.206		0.033		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	3	E1	0.554	0.553	0.089	0.089	12-Nov-09
			0.551		0.089		
			0.553		0.089		
		E2	0.225	0.223	0.036	0.036	
			0.224		0.036		
			0.219		0.035		
		E3	0.219	0.221	0.035	0.036	
			0.220		0.035		
			0.223		0.036		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	4	E1	0.711	0.713	0.115	0.115	13-Nov-09
			0.712		0.115		
			0.715		0.115		
		E2	0.223	0.222	0.036	0.036	
			0.221		0.036		
			0.223		0.036		
		E3	0.214	0.214	0.035	0.035	
			0.213		0.034		
			0.216		0.035		
Tap Water w/ Charcoal Filter &	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date

5 g/L Cal-Mag	5	E1	0.903	0.904	0.146	0.146	14-Nov-09
			0.905		0.146		
			0.903		0.146		
		E2	0.408	0.410	0.066	0.066	
			0.410		0.066		
			0.411		0.066		
		E3	0.236	0.238	0.038	0.038	
			0.239		0.039		
			0.238		0.038		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	6	E1	0.903	0.905	0.146	0.146	15-Nov-09
			0.905		0.146		
			0.907		0.146		
		E2	0.518	0.518	0.084	0.084	
			0.520		0.084		
			0.517		0.083		
		E3	0.221	0.220	0.036	0.036	
			0.220		0.035		
			0.220		0.035		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	7	E1	2.340	2.347	0.377	0.379	16-Nov-09
			2.350		0.379		
			2.350		0.379		
		E2	0.660	0.670	0.106	0.108	
			0.670		0.108		
			0.680		0.110		
		E3	0.199	0.199	0.032	0.032	
			0.198		0.032		
			0.200		0.032		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	8	E1	2.560	2.550	0.413	0.411	17-Nov-09
			2.530		0.408		
			2.560		0.413		
		E2	1.230	1.227	0.198	0.198	
			1.200		0.194		
			1.250		0.202		
		E3	0.246	0.245	0.040	0.039	
			0.243		0.039		
			0.245		0.040		
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	E1	2.160	2.160	0.348	0.348	18-Nov-09

			2.180		0.352		
			2.140		0.345		
		E2	1.200	1.207	0.194	0.195	
			1.200		0.194		
			1.220		0.197		
		E3	0.335	0.335	0.054	0.054	
			0.336		0.054		
			0.335		0.054		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	10	E1	2.700	2.700	0.436	0.436	19-Nov-09
			2.720		0.439		
			2.680		0.432		
		E2	1.600	1.613	0.258	0.260	
			1.620		0.261		
			1.620		0.261		
		E3	0.620	0.633	0.100	0.102	
			0.640		0.103		
			0.640		0.103		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	11	E1	5.860	5.873	0.945	0.947	20-Nov-09
			5.860		0.945		
			5.900		0.952		
		E2	2.960	2.953	0.477	0.476	
			2.980		0.481		
			2.920		0.471		
		E3	1.420	1.420	0.229	0.229	
			1.420		0.229		
			1.420		0.229		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	12	E1	4.680	4.680	0.755	0.755	21-Nov-09
			4.680		0.755		
			4.680		0.755		
		E2	3.100	3.127	0.500	0.504	
			3.120		0.503		
			3.160		0.510		
		E3	1.740	1.747	0.281	0.282	
			1.780		0.287		
			1.720		0.277		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Tap Water w/ Charcoal Filter & 5 g/L Cal-Mag	13	E1	6.320	6.313	1.019	1.018	22-Nov-09
			6.300		1.016		
			6.320		1.019		

		E2	3.800	3.807	0.613	0.614	
			3.800		0.613		
			3.820		0.616		
		E3	2.300	2.307	0.371	0.372	
			2.280		0.368		
			2.340		0.377		

Appendix E: Experiment 3 Data

Table E.1: Data for D.I. Water w/ $A550_0 \sim 0.200$.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	0	A1	0.192	0.192	0.039	0.039	30-Jul-09
			0.192		0.039		
			0.192		0.039		
		A2	0.198	0.198	0.041	0.041	
			0.198		0.041		
			0.198		0.041		
		A3	0.195	0.195	0.040	0.040	
			0.195		0.040		
			0.195		0.040		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	1	A1	0.421	0.422	0.087	0.087	31-Jul-09
			0.421		0.087		
			0.423		0.087		
		A2	0.463	0.460	0.095	0.095	
			0.459		0.094		
			0.458		0.094		
		A3	0.438	0.436	0.090	0.090	
			0.436		0.090		
			0.433		0.089		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	2	A1	0.930	0.929	0.191	0.191	1-Aug-09
			0.927		0.191		
			0.930		0.191		
		A2	0.940	0.943	0.193	0.194	
			0.947		0.195		
			0.943		0.194		
		A3	0.862	0.862	0.177	0.177	
			0.863		0.178		
			0.862		0.177		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	3	A1	1.381	1.383	0.284	0.284	2-Aug-09
			1.384		0.285		
			1.383		0.284		
		A2	1.565	1.567	0.322	0.322	
			1.568		0.323		

			1.568		0.323		
		A3	1.523	1.522	0.313	0.313	
			1.519		0.312		
			1.525		0.314		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	4	A1	1.823	1.822	0.375	0.375	3-Aug-09
			1.820		0.374		
			1.823		0.375		
		A2	1.878	1.877	0.386	0.386	
			1.881		0.387		
			1.871		0.385		
		A3	1.848	1.853	0.380	0.381	
			1.852		0.381		
			1.858		0.382		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	5	A1	3.690	3.697	0.759	0.760	4-Aug-09
			3.710		0.763		
			3.690		0.759		
		A2	4.100	4.107	0.843	0.845	
			4.115		0.846		
			4.105		0.844		
		A3	4.230	4.220	0.870	0.868	
			4.200		0.864		
			4.230		0.870		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	6	A1	4.065	4.062	0.836	0.835	5-Aug-09
			4.055		0.834		
			4.065		0.836		
		A2	4.435	4.453	0.912	0.916	
			4.470		0.919		
			4.455		0.916		
		A3	4.605	4.603	0.947	0.947	
			4.605		0.947		
			4.600		0.946		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	7	A1	5.060	5.047	1.041	1.038	6-Aug-09
			5.040		1.037		
			5.040		1.037		
		A2	5.240	5.247	1.078	1.079	
			5.250		1.080		

			5.250		1.080		
		A3	5.750	5.763	1.183	1.186	
			5.750		1.183		
			5.790		1.191		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	8	A1	5.130	5.163	1.055	1.062	7-Aug-09
			5.160		1.061		
			5.200		1.070		
		A2	5.480	5.503	1.127	1.132	
			5.520		1.135		
			5.510		1.133		
		A3	6.020	6.100	1.238	1.255	
			6.130		1.261		
			6.150		1.265		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	9	A1	5.700	5.767	1.172	1.186	8-Aug-09
			5.810		1.195		
			5.790		1.191		
		A2	5.900	5.800	1.214	1.193	
			5.720		1.177		
			5.780		1.189		
		A3	6.730	6.780	1.384	1.395	
			6.790		1.397		
			6.820		1.403		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	10	A1	6.020	6.003	1.238	1.235	9-Aug-09
			5.980		1.230		
			6.010		1.236		
		A2	5.890	5.940	1.212	1.222	
			6.020		1.238		
			5.910		1.216		
		A3	7.040	7.130	1.448	1.467	
			7.200		1.481		
			7.150		1.471		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	11	A1	6.200	6.210	1.275	1.277	10-Aug-09
			6.210		1.277		
			6.220		1.279		
		A2	6.040	6.060	1.242	1.247	
			6.070		1.249		

			6.070		1.249		
		A3	7.650	7.650	1.574	1.574	
			7.650		1.574		
			7.650		1.574		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	12	A1	6.620	6.643	1.362	1.367	11-Aug-09
			6.640		1.366		
			6.670		1.372		
		A2	6.530	6.443	1.343	1.325	
			6.440		1.325		
			6.360		1.308		
		A3	8.180	8.180	1.683	1.683	
			8.180		1.683		
			8.180		1.683		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	13	A1	6.990	7.023	1.438	1.445	12-Aug-09
			7.050		1.450		
			7.030		1.446		
		A2	6.510	6.510	1.339	1.339	
			6.490		1.335		
			6.530		1.343		
		A3	9.130	9.180	1.878	1.888	
			9.200		1.892		
			9.210		1.894		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	14	A1	7.840	7.777	1.613	1.600	13-Aug-09
			7.760		1.596		
			7.730		1.590		
		A2	7.430	7.407	1.528	1.524	
			7.390		1.520		
			7.400		1.522		
		A3	10.700	10.693	2.201	2.200	
			10.700		2.201		
			10.680		2.197		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	15	A1	9.600	9.660	1.975	1.987	14-Aug-09
			9.620		1.979		
			9.760		2.008		
		A2	7.660	7.707	1.576	1.585	
			7.740		1.592		

			7.720		1.588		
		A3	10.940	11.027	2.250	2.268	
			11.120		2.287		
			11.020		2.267		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	16	A1	10.760	10.847	2.213	2.231	15-Aug-09
			10.900		2.242		
			10.880		2.238		
		A2	7.800	7.827	1.604	1.610	
			7.840		1.613		
			7.840		1.613		
		A3	13.120	13.140	2.699	2.703	
			13.120		2.699		
			13.180		2.711		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	17	A1	11.100	11.153	2.283	2.294	16-Aug-09
			11.140		2.291		
			11.220		2.308		
		A2	8.380	8.333	1.724	1.714	
			8.300		1.707		
			8.320		1.711		
		A3	14.200	14.240	2.921	2.929	
			14.240		2.929		
			14.280		2.937		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 2.0 mL of algae Solution	18	A1	12.700	12.587	2.612	2.589	17-Aug-09
			12.520		2.575		
			12.540		2.579		
		A2	8.400	8.340	1.728	1.716	
			8.320		1.711		
			8.300		1.707		
		A3	18.700	18.680	3.847	3.842	
			18.680		3.842		
			18.660		3.838		

Table E.2: Data for D.I. Water w/ $A550_0 \sim 0.100$.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 1.0 mL of algae Solution	0	B1	0.104	0.104	0.021	0.021	30-Jul-09
			0.104		0.021		

			0.104		0.021		
		B2	0.101	0.101	0.021	0.021	
			0.101		0.021		
			0.101		0.021		
		B3	0.104	0.104	0.021	0.021	
			0.104		0.021		
			0.104		0.021		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	1	B1	0.250	0.251	0.051	0.052	31-Jul-09
			0.250		0.051		
			0.252		0.052		
		B2	0.247	0.247	0.051	0.051	
			0.247		0.051		
			0.247		0.051		
		B3	0.236	0.234	0.049	0.048	
			0.233		0.048		
			0.234		0.048		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	2	B1	0.642	0.643	0.132	0.132	1-Aug-09
			0.645		0.133		
			0.643		0.132		
		B2	0.581	0.577	0.120	0.119	
			0.573		0.118		
			0.576		0.118		
		B3	0.614	0.616	0.126	0.127	
			0.617		0.127		
			0.617		0.127		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	3	B1	0.876	0.877	0.180	0.180	2-Aug-09
			0.878		0.181		
			0.878		0.181		
		B2	1.074	1.074	0.221	0.221	
			1.073		0.221		
			1.074		0.221		
		B3	1.082	1.080	0.223	0.222	
			1.080		0.222		
			1.077		0.222		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	4	B1	1.104	1.102	0.227	0.227	3-Aug-09
			1.102		0.227		

			1.100		0.226		
		B2	1.434	1.441	0.295	0.296	
			1.443		0.297		
			1.445		0.297		
		B3	1.780	1.776	0.366	0.365	
			1.775		0.365		
			1.772		0.365		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	5	B1	2.065	2.067	0.425	0.425	4-Aug-09
			2.070		0.426		
			2.065		0.425		
		B2	3.110	3.108	0.640	0.639	
			3.105		0.639		
			3.110		0.640		
		B3	3.690	3.702	0.759	0.761	
			3.705		0.762		
			3.710		0.763		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	6	B1	2.695	2.713	0.554	0.558	5-Aug-09
			2.735		0.563		
			2.710		0.557		
		B2	3.450	3.468	0.710	0.713	
			3.475		0.715		
			3.480		0.716		
		B3	4.150	4.133	0.854	0.850	
			4.115		0.846		
			4.135		0.851		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	7	B1	3.670	3.687	0.755	0.758	6-Aug-09
			3.690		0.759		
			3.700		0.761		
		B2	4.710	4.723	0.969	0.972	
			4.740		0.975		
			4.720		0.971		
		B3	5.270	5.270	1.084	1.084	
			5.270		1.084		
			5.270		1.084		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	8	B1	4.160	4.147	0.856	0.853	7-Aug-09
			4.140		0.852		

			4.140		0.852		
		B2	4.850	4.873	0.998	1.002	
			4.920		1.012		
			4.850		0.998		
		B3	5.660	5.610	1.164	1.154	
			5.580		1.148		
			5.590		1.150		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	9	B1	4.600	4.557	0.946	0.937	8-Aug-09
			4.530		0.932		
			4.540		0.934		
		B2	5.090	5.083	1.047	1.046	
			5.080		1.045		
			5.080		1.045		
		B3	5.750	5.750	1.183	1.183	
			5.750		1.183		
			5.750		1.183		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	10	B1	5.210	5.260	1.072	1.082	9-Aug-09
			5.260		1.082		
			5.310		1.092		
		B2	5.210	5.223	1.072	1.074	
			5.230		1.076		
			5.230		1.076		
		B3	6.490	6.527	1.335	1.343	
			6.580		1.354		
			6.510		1.339		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	11	B1	5.500	5.557	1.131	1.143	10-Aug-09
			5.590		1.150		
			5.580		1.148		
		B2	5.590	5.493	1.150	1.130	
			5.430		1.117		
			5.460		1.123		
		B3	6.770	6.800	1.393	1.399	
			6.810		1.401		
			6.820		1.403		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	12	B1	6.150	6.177	1.265	1.271	11-Aug-09
			6.180		1.271		

			6.200		1.275		
		B2	5.830	5.793	1.199	1.192	
			5.760		1.185		
			5.790		1.191		
		B3	7.570	7.493	1.557	1.541	
			7.490		1.541		
			7.420		1.526		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	13	B1	6.820	6.883	1.403	1.416	12-Aug-09
			6.930		1.426		
			6.900		1.419		
		B2	6.070	6.077	1.249	1.250	
			6.080		1.251		
			6.080		1.251		
		B3	7.910	7.877	1.627	1.620	
			7.890		1.623		
			7.830		1.611		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	14	B1	8.800	7.850	1.810	1.615	13-Aug-09
			8.870		1.825		
			5.879		1.209		
		B2	7.310	7.330	1.504	1.508	
			7.330		1.508		
			7.350		1.512		
		B3	8.510	8.563	1.751	1.761	
			8.620		1.773		
			8.560		1.761		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	15	B1	12.020	12.067	2.473	2.482	14-Aug-09
			12.100		2.489		
			12.080		2.485		
		B2	8.800	8.773	1.810	1.805	
			8.760		1.802		
			8.760		1.802		
		B3	10.040	10.027	2.065	2.062	
			10.040		2.065		
			10.000		2.057		
DI Water w/ 1.0 mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	16	B1	13.520	13.533	2.781	2.784	15-Aug-09
			13.480		2.773		

			13.600		2.798		
		B2	9.200	9.313	1.892	1.916	
			9.380		1.929		
			9.360		1.925		
		B3	10.660	10.667	2.193	2.194	
			10.660		2.193		
			10.680		2.197		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
DI Water w/ 1.0 mL of algae Solution	17	B1	17.700	17.633	3.641	3.627	16-Aug-09
			17.580		3.616		
			17.620		3.624		
		B2	10.520	10.560	2.164	2.172	
			10.580		2.176		
			10.580		2.176		
		B3	11.160	11.167	2.296	2.297	
			11.220		2.308		
			11.120		2.287		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
DI Water w/ 1.0 mL of algae Solution	18	B1	19.580	19.527	4.028	4.017	17-Aug-09
			19.460		4.003		
			19.540		4.019		
		B2	11.280	11.407	2.320	2.346	
			11.460		2.357		
			11.480		2.361		
		B3	11.920	11.907	2.452	2.449	
			11.920		2.452		
			11.880		2.444		

Table E.3: Data for D.I. Water w/ $A550_0 \sim 0.050$.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	0	C1	0.054	0.054	0.011	0.011	30-Jul-09
			0.054		0.011		
			0.054		0.011		
		C2	0.054	0.054	0.011	0.011	
			0.054		0.011		
			0.054		0.011		
		C3	0.052	0.052	0.011	0.011	
			0.052		0.011		
			0.052		0.011		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	1	C1	0.117	0.120	0.024	0.025	31-Jul-09
			0.121		0.025		
			0.122		0.025		
		C2	0.129	0.131	0.027	0.027	
			0.133		0.027		
			0.131		0.027		
		C3	0.123	0.123	0.025	0.025	
			0.123		0.025		
			0.123		0.025		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	2	C1	0.310	0.311	0.064	0.064	1-Aug-09
			0.312		0.064		
			0.312		0.064		
		C2	0.364	0.367	0.075	0.075	
			0.369		0.076		
			0.368		0.076		
		C3	0.280	0.277	0.058	0.057	
			0.276		0.057		
			0.274		0.056		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	3	C1	0.716	0.714	0.147	0.147	2-Aug-09
			0.712		0.146		
			0.715		0.147		
		C2	0.819	0.819	0.168	0.168	
			0.818		0.168		
			0.820		0.169		
		C3	0.675	0.675	0.139	0.139	
			0.675		0.139		
			0.675		0.139		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	4	C1	1.063	1.060	0.219	0.218	3-Aug-09
			1.057		0.217		
			1.059		0.218		
		C2	1.469	1.466	0.302	0.302	
			1.470		0.302		
			1.460		0.300		
		C3	1.335	1.336	0.275	0.275	
			1.338		0.275		
			1.336		0.275		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	5	C1	2.160	2.162	0.444	0.445	4-Aug-09
			2.155		0.443		
			2.170		0.446		
		C2	3.005	3.002	0.618	0.617	
			3.005		0.618		
			2.995		0.616		
		C3	2.800	2.812	0.576	0.578	
			2.825		0.581		
			2.810		0.578		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	6	C1	2.790	2.785	0.574	0.573	5-Aug-09
			2.770		0.570		
			2.795		0.575		
		C2	3.555	3.520	0.731	0.724	
			3.495		0.719		
			3.510		0.722		
		C3	3.445	3.438	0.709	0.707	
			3.425		0.705		
			3.445		0.709		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	7	C1	3.960	3.963	0.815	0.815	6-Aug-09
			3.980		0.819		
			3.950		0.813		
		C2	4.730	4.740	0.973	0.975	
			4.740		0.975		
			4.750		0.977		
		C3	4.540	4.560	0.934	0.938	
			4.570		0.940		
			4.570		0.940		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	8	C1	4.250	4.203	0.874	0.865	7-Aug-09
			4.190		0.862		
			4.170		0.858		
		C2	5.350	5.107	1.100	1.050	
			4.990		1.026		
			4.980		1.024		
		C3	5.346	4.869	1.100	1.002	
			4.650		0.957		
			4.610		0.948		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	9	C1	4.720	4.747	0.971	0.976	8-Aug-09
			4.760		0.979		
			4.760		0.979		
		C2	5.540	5.517	1.140	1.135	
			5.500		1.131		
			5.510		1.133		
		C3	5.580	5.597	1.148	1.151	
			5.600		1.152		
			5.610		1.154		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	10	C1	5.130	5.113	1.055	1.052	9-Aug-09
			5.090		1.047		
			5.120		1.053		
		C2	5.750	5.703	1.183	1.173	
			5.660		1.164		
			5.700		1.172		
		C3	6.020	6.023	1.238	1.239	
			6.020		1.238		
			6.030		1.240		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	11	C1	5.540	5.517	1.140	1.135	10-Aug-09
			5.510		1.133		
			5.500		1.131		
		C2	6.040	6.067	1.242	1.248	
			6.100		1.255		
			6.060		1.247		
		C3	6.610	6.663	1.360	1.371	
			6.680		1.374		
			6.700		1.378		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	12	C1	6.210	6.203	1.277	1.276	11-Aug-09
			6.190		1.273		
			6.210		1.277		
		C2	6.690	6.637	1.376	1.365	
			6.590		1.356		
			6.630		1.364		
		C3	7.160	7.203	1.473	1.482	
			7.250		1.491		
			7.200		1.481		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	13	C1	6.800	6.853	1.399	1.410	12-Aug-09
			6.890		1.417		
			6.870		1.413		
		C2	7.040	7.040	1.448	1.448	
			7.040		1.448		
			7.040		1.448		
		C3	8.260	8.163	1.699	1.679	
			8.130		1.672		
			8.100		1.666		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	14	C1	7.510	7.563	1.545	1.556	13-Aug-09
			7.600		1.563		
			7.580		1.559		
		C2	7.790	7.743	1.602	1.593	
			7.710		1.586		
			7.730		1.590		
		C3	8.150	8.150	1.676	1.676	
			8.130		1.672		
			8.170		1.681		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	15	C1	10.960	11.047	2.254	2.272	14-Aug-09
			11.122		2.288		
			11.060		2.275		
		C2	9.800	9.847	2.016	2.025	
			9.920		2.041		
			9.820		2.020		
		C3	10.200	9.953	2.098	2.047	
			9.860		2.028		
			9.800		2.016		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	16	C1	13.400	13.440	2.756	2.765	15-Aug-09
			13.420		2.760		
			13.500		2.777		
		C2	11.060	11.635	2.275	2.393	
			10.760		2.213		
			13.084		2.691		
		C3	12.480	12.693	2.567	2.611	
			12.820		2.637		
			12.780		2.629		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	17	C1	17.160	17.073	3.530	3.512	16-Aug-09
			17.020		3.501		
			17.040		3.505		
		C2	12.280	12.153	2.526	2.500	
			12.100		2.489		
			12.080		2.485		
		C3	13.900	13.893	2.859	2.858	
			13.900		2.859		
			13.880		2.855		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.50 mL of algae Solution	18	C1	22.200	22.140	4.567	4.554	17-Aug-09
			22.160		4.558		
			22.060		4.538		
		C2	13.560	13.513	2.789	2.780	
			13.480		2.773		
			13.500		2.777		
		C3	15.100	14.993	3.106	3.084	
			14.920		3.069		
			14.960		3.077		

Table E.4: Data for D.I. Water w/ $A550_0 \sim 0.025$.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	0	D1	0.027	0.027	0.006	0.006	30-Jul-09
			0.027		0.006		
			0.027		0.006		
		D2	0.027	0.027	0.006	0.006	
			0.027		0.006		
			0.027		0.006		
		D3	0.027	0.027	0.006	0.006	
			0.027		0.006		
			0.027		0.006		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	1	D1	0.060	0.066	0.012	0.014	31-Jul-09
			0.068		0.014		
			0.069		0.014		
		D2	0.069	0.070	0.014	0.014	
			0.070		0.014		
			0.070		0.014		

		D3	0.069	0.070	0.014	0.014	
			0.068		0.014		
			0.072		0.015		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	2	D1	0.198	0.199	0.041	0.041	1-Aug-09
			0.199		0.041		
			0.199		0.041		
		D2	0.200	0.197	0.041	0.040	
			0.196		0.040		
			0.194		0.040		
		D3	0.210	0.210	0.043	0.043	
			0.211		0.043		
			0.209		0.043		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	3	D1	0.490	0.492	0.101	0.101	2-Aug-09
			0.494		0.102		
			0.491		0.101		
		D2	0.517	0.519	0.106	0.107	
			0.522		0.107		
			0.518		0.107		
		D3	0.378	0.377	0.078	0.078	
			0.378		0.078		
			0.375		0.077		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	4	D1	1.316	1.315	0.271	0.271	3-Aug-09
			1.317		0.271		
			1.313		0.270		
		D2	0.924	0.920	0.190	0.189	
			0.915		0.188		
			0.922		0.190		
		D3	0.777	0.772	0.160	0.159	
			0.768		0.158		
			0.772		0.159		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	5	D1	2.990	2.993	0.615	0.616	4-Aug-09
			2.990		0.615		
			3.000		0.617		
		D2	2.115	2.117	0.435	0.435	
			2.120		0.436		
			2.115		0.435		

		D3	1.760	1.757	0.362	0.361	
			1.755		0.361		
			1.755		0.361		
DI Water w/ 0.25mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	D1	3.465	3.490	0.713	0.718	5-Aug-09
			3.515		0.723		
			3.490		0.718		
		D2	2.835	2.847	0.583	0.586	
			2.855		0.587		
			2.850		0.586		
		D3	2.725	2.717	0.561	0.559	
			2.710		0.557		
			2.715		0.558		
DI Water w/ 0.25mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	7	D1	4.510	4.490	0.928	0.924	6-Aug-09
			4.480		0.922		
			4.480		0.922		
		D2	3.980	4.000	0.819	0.823	
			3.990		0.821		
			4.030		0.829		
		D3	3.880	3.870	0.798	0.796	
			3.860		0.794		
			3.870		0.796		
DI Water w/ 0.25mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	D1	4.570	4.567	0.940	0.939	7-Aug-09
			4.540		0.934		
			4.590		0.944		
		D2	4.250	4.203	0.874	0.865	
			4.170		0.858		
			4.190		0.862		
		D3	4.030	3.993	0.829	0.821	
			3.950		0.813		
			4.000		0.823		
DI Water w/ 0.25mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	D1	5.090	5.120	1.047	1.053	8-Aug-09
			5.140		1.057		
			5.130		1.055		
		D2	5.190	5.157	1.068	1.061	
			5.140		1.057		
			5.140		1.057		

		D3	4.840	4.853	0.996	0.998	
			4.860		1.000		
			4.860		1.000		
DI Water w/ 0.25mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	10	D1	5.540	5.520	1.140	1.135	9-Aug-09
			5.510		1.133		
			5.510		1.133		
		D2	5.650	5.660	1.162	1.164	
			5.670		1.166		
			5.660		1.164		
		D3	5.200	5.263	1.070	1.083	
			5.310		1.092		
			5.280		1.086		
DI Water w/ 0.25mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	11	D1	5.820	5.820	1.197	1.197	10-Aug-09
			5.820		1.197		
			5.820		1.197		
		D2	6.250	6.197	1.286	1.275	
			6.190		1.273		
			6.150		1.265		
		D3	5.770	5.833	1.187	1.200	
			5.890		1.212		
			5.840		1.201		
DI Water w/ 0.25mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	12	D1	6.510	6.510	1.339	1.339	11-Aug-09
			6.510		1.339		
			6.510		1.339		
		D2	6.800	6.800	1.399	1.399	
			6.800		1.399		
			6.800		1.399		
		D3	6.310	6.283	1.298	1.292	
			6.300		1.296		
			6.240		1.284		
DI Water w/ 0.25mL of algae Solution	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	13	D1	6.800	6.810	1.399	1.401	12-Aug-09
			6.800		1.399		
			6.830		1.405		
		D2	7.930	7.877	1.631	1.620	
			7.840		1.613		
			7.860		1.617		

		D3	7.030	6.987	1.446	1.437	
			6.960		1.432		
			6.970		1.434		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	14	D1	7.350	7.440	1.512	1.530	13-Aug-09
			7.510		1.545		
			7.460		1.535		
		D2	8.230	8.233	1.693	1.694	
			8.280		1.703		
			8.190		1.685		
		D3	8.010	8.070	1.648	1.660	
			8.080		1.662		
			8.120		1.670		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	15	D1	9.280	9.333	1.909	1.920	14-Aug-09
			9.440		1.942		
			9.280		1.909		
		D2	9.980	9.980	2.053	2.053	
			9.980		2.053		
			9.980		2.053		
		D3	9.560	9.527	1.966	1.960	
			9.520		1.958		
			9.500		1.954		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	16	D1	10.300	10.220	2.119	2.102	15-Aug-09
			10.200		2.098		
			10.160		2.090		
		D2	12.020	12.020	2.473	2.473	
			12.020		2.473		
			12.020		2.473		
		D3	11.500	11.427	2.366	2.350	
			11.420		2.349		
			11.360		2.337		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	17	D1	11.080	11.133	2.279	2.290	16-Aug-09
			11.160		2.296		
			11.160		2.296		
		D2	13.740	13.727	2.826	2.824	
			13.720		2.822		
			13.720		2.822		

		D3	13.200	13.207	2.715	2.717	
			13.160		2.707		
			13.260		2.728		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
DI Water w/ 0.25mL of algae Solution	18	D1	12.040	12.193	2.477	2.508	17-Aug-09
			12.240		2.518		
			12.300		2.530		
		D2	18.960	18.980	3.900	3.904	
			19.160		3.941		
			18.820		3.871		
		D3	16.580	16.493	3.411	3.393	
			16.440		3.382		
			16.460		3.386		

Appendix F: Experiment 4 Data

Table F.1: Data for Culture using $NaNO_3$ as "N".

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	0	A1	0.184	0.185	0.029	0.029	24-Aug-09
			0.184		0.029		
			0.186		0.030		
		A2	0.182	0.183	0.029	0.029	
			0.184		0.029		
			0.184		0.029		
		A3	0.199	0.199	0.032	0.032	
			0.199		0.032		
			0.198		0.032		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	1	A1	0.403	0.400	0.064	0.064	25-Aug-09
			0.398		0.063		
			0.399		0.064		
		A2	0.461	0.462	0.073	0.074	
			0.463		0.074		
			0.462		0.074		
		A3	0.520	0.518	0.083	0.083	
			0.517		0.082		
			0.517		0.082		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	2	A1	0.713	0.710	0.114	0.113	26-Aug-09
			0.708		0.113		
			0.710		0.113		
		A2	0.808	0.806	0.129	0.128	
			0.804		0.128		
			0.805		0.128		
		A3	0.792	0.790	0.126	0.126	
			0.791		0.126		
			0.788		0.126		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	3	A1	1.608	1.609	0.256	0.256	27-Aug-09
			1.608		0.256		
			1.610		0.257		
		A2	1.578	1.569	0.252	0.250	
			1.564		0.249		
			1.566		0.250		

		A3	1.556	1.553	0.248	0.248	
			1.550		0.247		
			1.554		0.248		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	4	A1	2.820	2.807	0.450	0.447	28-Aug-09
			2.820		0.450		
			2.780		0.443		
		A2	2.870	2.853	0.457	0.455	
			2.850		0.454		
			2.840		0.453		
		A3	3.180	3.190	0.507	0.508	
			3.190		0.508		
			3.200		0.510		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	5	A1	3.130	3.130	0.499	0.499	29-Aug-09
			3.130		0.499		
			3.130		0.499		
		A2	3.625	3.625	0.578	0.578	
			3.625		0.578		
			3.625		0.578		
		A3	4.231	4.231	0.674	0.674	
			4.231		0.674		
			4.231		0.674		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	6	A1	3.460	3.473	0.552	0.554	30-Aug-09
			3.480		0.555		
			3.480		0.555		
		A2	4.940	4.940	0.787	0.787	
			4.940		0.787		
			4.940		0.787		
		A3	5.830	5.830	0.929	0.929	
			5.830		0.929		
			5.830		0.929		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	7	A1	4.660	4.760	0.743	0.759	31-Aug-09
			4.840		0.771		
			4.780		0.762		
		A2	5.980	5.980	0.953	0.953	
			5.980		0.953		
			5.980		0.953		

		A3	8.400	8.400	1.339	1.339	
			8.400		1.339		
			8.400		1.339		
BBM4N ($NaNO_3$)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	A1	5.100	5.100	0.813	0.813	1-Sep-09
			5.100		0.813		
			5.100		0.813		
		A2	6.640	6.640	1.058	1.058	
			6.640		1.058		
			6.640		1.058		
		A3	9.010	9.010	1.436	1.436	
			9.010		1.436		
			9.010		1.436		
BBM4N ($NaNO_3$)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	A1	6.620	6.620	1.055	1.055	2-Sep-09
			6.620		1.055		
			6.620		1.055		
		A2	8.460	8.460	1.349	1.349	
			8.460		1.349		
			8.460		1.349		
		A3	4.760	4.760	0.759	0.759	
			4.760		0.759		
			4.760		0.759		
BBM4N ($NaNO_3$)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	10	A1	8.220	8.220	1.310	1.310	3-Sep-09
			8.220		1.310		
			8.220		1.310		
		A2	9.540	9.540	1.521	1.521	
			9.540		1.521		
			9.540		1.521		
		A3	5.660	5.660	0.902	0.902	
			5.660		0.902		
			5.660		0.902		

Table F.2: Data for Culture using $(NH_4)_2SO_4$ as "N".

BBM4N $(NH_4)_2SO_4$	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	0	B1	0.193	0.193	0.031	0.031	24-Aug-09

			0.193		0.031		
			0.193		0.031		
		B2	0.192	0.190	0.031	0.030	
			0.188		0.030		
			0.190		0.030		
		B3	0.204	0.205	0.033	0.033	
			0.205		0.033		
			0.206		0.033		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	1	B1	0.515	0.517	0.082	0.082	25-Aug-09
			0.518		0.083		
			0.519		0.083		
		B2	0.460	0.461	0.073	0.073	
			0.461		0.073		
			0.461		0.073		
		B3	0.449	0.449	0.072	0.072	
			0.450		0.072		
			0.449		0.072		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	2	B1	0.992	0.989	0.158	0.158	26-Aug-09
			0.988		0.157		
			0.987		0.157		
		B2	0.730	0.734	0.116	0.117	
			0.736		0.117		
			0.737		0.117		
		B3	0.793	0.793	0.126	0.126	
			0.793		0.126		
			0.794		0.127		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	3	B1	1.268	1.271	0.202	0.203	27-Aug-09
			1.270		0.202		
			1.274		0.203		
		B2	0.894	0.901	0.143	0.144	
			0.910		0.145		
			0.898		0.143		
		B3	1.210	1.204	0.193	0.192	
			1.196		0.191		
			1.206		0.192		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	4	B1	1.600	1.627	0.255	0.259	28-Aug-09
			1.630		0.260		
			1.650		0.263		
		B2	0.840	0.867	0.134	0.138	
			0.870		0.139		
			0.890		0.142		
		B3	1.530	1.540	0.244	0.245	
			1.530		0.244		
			1.560		0.249		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	5	B1	1.213	1.213	0.193	0.193	29-Aug-09
			1.213		0.193		
			1.213		0.193		
		B2	0.730	0.730	0.116	0.116	
			0.730		0.116		
			0.730		0.116		
		B3	1.730	1.730	0.276	0.276	
			1.730		0.276		
			1.730		0.276		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	6	B1	0.660	0.667	0.105	0.106	30-Aug-09
			0.680		0.108		
			0.660		0.105		
		B2	0.680	0.680	0.108	0.108	
			0.680		0.108		
			0.680		0.108		
		B3	2.020	2.017	0.322	0.321	
			2.020		0.322		
			2.010		0.320		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	7	B1	0.920	0.920	0.147	0.147	31-Aug-09
			0.920		0.147		
			0.920		0.147		
		B2	1.120	1.120	0.179	0.179	
			1.120		0.179		
			1.120		0.179		
		B3	2.440	2.440	0.389	0.389	
			2.440		0.389		

			2.440		0.389		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	8	B1	0.930	0.930	0.148	0.148	1-Sep-09
			0.930		0.148		
			0.930		0.148		
		B2	0.712	0.712	0.113	0.113	
			0.712		0.113		
			0.712		0.113		
		B3	1.066	1.066	0.170	0.170	
			1.066		0.170		
			1.066		0.170		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	9	B1	0.764	0.764	0.122	0.122	2-Sep-09
			0.764		0.122		
			0.764		0.122		
		B2	0.619	0.619	0.099	0.099	
			0.619		0.099		
			0.619		0.099		
		B3	1.059	1.059	0.169	0.169	
			1.059		0.169		
			1.059		0.169		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	10	B1	0.693	0.693	0.110	0.110	3-Sep-09
			0.693		0.110		
			0.693		0.110		
		B2	0.630	0.630	0.100	0.100	
			0.630		0.100		
			0.630		0.100		
		B3	1.107	1.107	0.176	0.176	
			1.107		0.176		
			1.107		0.176		

Table F.3: Data for Culture using NH_4NO_3 as "N".

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	0	C1	0.201	0.201	0.032	0.032	24-Aug-09
			0.201		0.032		
			0.201		0.032		

		C2	0.206	0.204	0.033	0.032	
			0.203		0.032		
			0.202		0.032		
		C3	0.199	0.200	0.032	0.032	
			0.200		0.032		
			0.200		0.032		
BBM4N (NH_4NO_3)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	1	C1	0.434	0.435	0.069	0.069	25-Aug-09
			0.435		0.069		
			0.436		0.069		
		C2	0.478	0.477	0.076	0.076	
			0.477		0.076		
			0.475		0.076		
		C3	0.479	0.478	0.076	0.076	
			0.480		0.077		
			0.475		0.076		
BBM4N (NH_4NO_3)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	2	C1	0.705	0.706	0.112	0.113	26-Aug-09
			0.707		0.113		
			0.707		0.113		
		C2	0.703	0.705	0.112	0.112	
			0.706		0.113		
			0.705		0.112		
		C3	0.760	0.755	0.121	0.120	
			0.751		0.120		
			0.754		0.120		
BBM4N (NH_4NO_3)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	3	C1	1.030	1.034	0.164	0.165	27-Aug-09
			1.032		0.165		
			1.040		0.166		
		C2	0.970	0.970	0.155	0.155	
			0.970		0.155		
			0.970		0.155		
		C3	0.886	0.893	0.141	0.142	
			0.896		0.143		
			0.896		0.143		
BBM4N (NH_4NO_3)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	C1	1.030	1.033	0.164	0.165	28-Aug-09

			1.020		0.163		
			1.050		0.167		
		C2	0.970		0.155		
			1.000	0.973	0.159	0.155	
			0.950		0.151		
		C3	0.920		0.147		
			0.950	0.937	0.151	0.149	
			0.940		0.150		
BBM4N (NH_4NO_3)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		C1	0.836		0.133		
			0.838	0.837	0.134	0.133	
			0.836		0.133		
	5	C2	0.883		0.141		
			0.885	0.884	0.141	0.141	29-Aug-09
			0.885		0.141		
		C3	1.130		0.180		
			1.135	1.133	0.181	0.181	
			1.133		0.181		
BBM4N (NH_4NO_3)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		C1	0.680		0.108		
			0.680	0.680	0.108	0.108	
			0.680		0.108		
	6	C2	0.760		0.121		
			0.760	0.760	0.121	0.121	30-Aug-09
			0.760		0.121		
		C3	1.290		0.206		
			1.280	1.287	0.204	0.205	
			1.290		0.206		
BBM4N (NH_4NO_3)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		C1	1.140		0.182		
			1.140	1.140	0.182	0.182	
			1.140		0.182		
	7	C2	1.340		0.214		
			1.340	1.340	0.214	0.214	31-Aug-09
			1.340		0.214		
		C3	1.380		0.220		
			1.380	1.380	0.220	0.220	
			1.380		0.220		
BBM4N (NH_4NO_3)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date

	8	C1	0.946	0.946	0.151	0.151	1-Sep-09
			0.946		0.151		
			0.946		0.151		
		C2	1.188	1.188	0.189	0.189	
			1.188		0.189		
			1.188		0.189		
		C3	1.300	1.300	0.207	0.207	
			1.300		0.207		
			1.300		0.207		
BBM4N (NH_4NO_3)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	C1	0.970	0.970	0.155	0.155	2-Sep-09
			0.970		0.155		
			0.970		0.155		
		C2	1.150	1.150	0.183	0.183	
			1.150		0.183		
			1.150		0.183		
		C3	1.227	1.227	0.196	0.196	
			1.227		0.196		
			1.227		0.196		
BBM4N (NH_4NO_3)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	10	C1	0.924	0.924	0.147	0.147	3-Sep-09
			0.924		0.147		
			0.924		0.147		
		C2	1.197	1.197	0.191	0.191	
			1.197		0.191		
			1.197		0.191		
		C3	1.297	1.297	0.207	0.207	
			1.297		0.207		
			1.297		0.207		

Table F.4: Data for Culture using ACS Grade Urea as “N”.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (Urea)	0	D1	0.218	0.218	0.035	0.035	24-Aug-09
			0.218		0.035		
			0.218		0.035		
		D2	0.209	0.210	0.033	0.034	
			0.210		0.033		
			0.212		0.034		

		D3	0.204	0.204	0.033	0.033	
			0.204		0.033		
			0.204		0.033		
BBM4N (Urea)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	1	D1	0.480	0.482	0.077	0.077	25-Aug-09
			0.486		0.077		
			0.481		0.077		
		D2	0.443	0.443	0.071	0.071	
			0.444		0.071		
			0.442		0.070		
		D3	0.367	0.367	0.058	0.059	
			0.368		0.059		
			0.367		0.058		
BBM4N (Urea)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	2	D1	1.418	1.414	0.226	0.225	26-Aug-09
			1.411		0.225		
			1.413		0.225		
		D2	1.086	1.083	0.173	0.173	
			1.080		0.172		
			1.082		0.172		
		D3	1.016	1.019	0.162	0.162	
			1.022		0.163		
			1.018		0.162		
BBM4N (Urea)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	3	D1	2.206	2.215	0.352	0.353	27-Aug-09
			2.218		0.354		
			2.220		0.354		
		D2	2.434	2.441	0.388	0.389	
			2.444		0.390		
			2.446		0.390		
		D3	2.112	2.118	0.337	0.338	
			2.120		0.338		
			2.122		0.338		
BBM4N (Urea)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	D1	5.090	5.070	0.811	0.808	28-Aug-09
			5.050		0.805		
			5.070		0.808		
		D2	5.020	5.020	0.800	0.800	
			5.020		0.800		

			5.020		0.800		
		D3	3.810	3.810	0.607	0.607	
			3.830		0.611		
			3.790		0.604		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (Urea)	5	D1	7.760	7.727	1.237	1.232	29-Aug-09
			7.700		1.227		
			7.720		1.231		
		D2	7.900	7.960	1.259	1.269	
			7.960		1.269		
			8.020		1.278		
		D3	5.600	5.647	0.893	0.900	
			5.680		0.905		
			5.660		0.902		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (Urea)	6	D1	9.760	9.760	1.556	1.556	30-Aug-09
			9.760		1.556		
			9.760		1.556		
		D2	13.700	13.700	2.184	2.184	
			13.700		2.184		
			13.700		2.184		
		D3	8.920	8.920	1.422	1.422	
			8.920		1.422		
			8.920		1.422		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (Urea)	7	D1	11.760	11.760	1.875	1.875	31-Aug-09
			11.760		1.875		
			11.760		1.875		
		D2	14.660	14.660	2.337	2.337	
			14.660		2.337		
			14.660		2.337		
		D3	11.280	11.280	1.798	1.798	
			11.280		1.798		
			11.280		1.798		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (Urea)	8	D1	9.760	9.760	1.556	1.556	1-Sep-09
			9.760		1.556		
			9.760		1.556		

			12.700		2.024		
		D2	12.700	12.700	2.024	2.024	
			12.700		2.024		
			10.620		1.693		
		D3	10.620	10.620	1.693	1.693	
			10.620		1.693		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
			7.500		1.196		
		D1	7.500	7.500	1.196	1.196	
			7.500		1.196		
BBM4N (Urea)			10.580		1.686		
	9	D2	10.580	10.580	1.686	1.686	2-Sep-09
			10.580		1.686		
			10.040		1.600		
		D3	10.040	10.040	1.600	1.600	
			10.040		1.600		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
			7.900		1.259		
		D1	7.900	7.900	1.259	1.259	
			7.900		1.259		
BBM4N (Urea)			11.520		1.836		
	10	D2	11.520	11.520	1.836	1.836	3-Sep-09
			11.520		1.836		
			11.340		1.808		
		D3	11.340	11.340	1.808	1.808	
			11.340		1.808		

Table F.5: Data for Culture made up as BBM using Commercial Fertilizer & EDTA.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
			0.204		0.033		
		E1	0.205	0.204	0.033	0.033	
			0.203		0.032		
			0.207		0.033		
BBM4N (CF w/ EDTA STEM)	0	E2	0.207	0.207	0.033	0.033	25-Aug-09
			0.207		0.033		
			0.209		0.033		
		E3	0.209	0.209	0.033	0.033	
			0.209		0.033		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ EDTA STEM)	1	E1	0.407	0.409	0.065	0.065	26-Aug-09
			0.410		0.065		
			0.409		0.065		
		E2	0.357	0.357	0.057	0.057	
			0.357		0.057		
			0.357		0.057		
		E3	0.655	0.656	0.104	0.105	
			0.655		0.104		
			0.657		0.105		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ EDTA STEM)	2	E1	1.330	1.325	0.212	0.211	27-Aug-09
			1.322		0.211		
			1.324		0.211		
		E2	0.870	0.876	0.139	0.140	
			0.880		0.140		
			0.878		0.140		
		E3	1.786	1.786	0.285	0.285	
			1.786		0.285		
			1.786		0.285		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ EDTA STEM)	3	E1	2.650	2.643	0.422	0.421	28-Aug-09
			2.650		0.422		
			2.630		0.419		
		E2	2.070	2.050	0.330	0.327	
			2.040		0.325		
			2.040		0.325		
		E3	3.890	3.917	0.620	0.624	
			3.930		0.626		
			3.930		0.626		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ EDTA STEM)	4	E1	4.950	4.917	0.789	0.784	29-Aug-09
			4.910		0.783		
			4.890		0.779		
		E2	4.300	4.313	0.685	0.688	
			4.290		0.684		
			4.350		0.693		
		E3	4.950	4.893	0.789	0.780	
			4.890		0.779		
			4.840		0.771		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ EDTA STEM)	5	E1	7.140	7.140	1.138	1.138	30-Aug-09
			7.140		1.138		
			7.140		1.138		
		E2	6.340	6.340	1.011	1.011	
			6.340		1.011		
			6.340		1.011		
		E3	5.360	5.360	0.854	0.854	
			5.360		0.854		
			5.360		0.854		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ EDTA STEM)	6	E1	10.420	10.420	1.661	1.661	31-Aug-09
			10.420		1.661		
			10.420		1.661		
		E2	11.600	11.600	1.849	1.849	
			11.600		1.849		
			11.600		1.849		
		E3	7.060	7.060	1.125	1.125	
			7.060		1.125		
			7.060		1.125		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ EDTA STEM)	7	E1	16.620	16.620	2.649	2.649	1-Sep-09
			16.620		2.649		
			16.620		2.649		
		E2	11.440	11.440	1.824	1.824	
			11.440		1.824		
			11.440		1.824		
		E3	8.900	8.900	1.419	1.419	
			8.900		1.419		
			8.900		1.419		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ EDTA STEM)	8	E1	11.280	11.280	1.798	1.798	2-Sep-09
			11.280		1.798		
			11.280		1.798		
		E2	11.600	11.600	1.849	1.849	
			11.600		1.849		
			11.600		1.849		
		E3	7.780	7.780	1.240	1.240	
			7.780		1.240		
			7.780		1.240		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ EDTA STEM)	9	E1	15.700	15.700	2.503	2.503	3-Sep-09
			15.700		2.503		
			15.700		2.503		
		E2	13.040	13.040	2.079	2.079	
			13.040		2.079		
			13.040		2.079		
		E3	8.780	8.780	1.400	1.400	
			8.780		1.400		
			8.780		1.400		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ EDTA STEM)	10	E1	20.700	20.700	3.300	3.300	4-Sep-09
			20.700		3.300		
			20.700		3.300		
		E2	12.600	12.600	2.008	2.008	
			12.600		2.008		
			12.600		2.008		
		E3	10.400	10.400	1.658	1.658	
			10.400		1.658		
			10.400		1.658		

Table F.6: Data for Culture made up as BBM using Commercial Fertilizer & No EDTA.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	0	F1	0.206	0.206	0.033	0.033	25-Aug-09
			0.206		0.033		
			0.207		0.033		
		F2	0.207	0.207	0.033	0.033	
			0.205		0.033		
			0.208		0.033		
		F3	0.216	0.216	0.034	0.034	
			0.215		0.034		
			0.216		0.034		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	1	F1	0.443	0.441	0.071	0.070	26-Aug-09
			0.439		0.070		
			0.442		0.070		
		F2	0.544	0.545	0.087	0.087	
			0.547		0.087		

			0.545		0.087		
		F3	0.511	0.511	0.081	0.081	
			0.511		0.081		
			0.511		0.081		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	2	F1	1.112	1.103	0.177	0.176	27-Aug-09
			1.098		0.175		
			1.098		0.175		
		F2	1.764	1.764	0.281	0.281	
			1.766		0.282		
			1.762		0.281		
		F3	1.704	1.710	0.272	0.273	
			1.712		0.273		
			1.714		0.273		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	3	F1	2.820	2.797	0.450	0.446	28-Aug-09
			2.790		0.445		
			2.780		0.443		
		F2	3.850	3.840	0.614	0.612	
			3.850		0.614		
			3.820		0.609		
		F3	3.710	3.697	0.591	0.589	
			3.710		0.591		
			3.670		0.585		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	4	F1	4.010	4.040	0.639	0.644	29-Aug-09
			4.040		0.644		
			4.070		0.649		
		F2	4.340	4.350	0.692	0.693	
			4.340		0.692		
			4.370		0.697		
		F3	5.360	5.363	0.854	0.855	
			5.340		0.851		
			5.390		0.859		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	5	F1	6.080	6.080	0.969	0.969	30-Aug-09
			6.080		0.969		
			6.080		0.969		
		F2	5.920	5.920	0.944	0.944	
			5.920		0.944		

			5.920		0.944		
		F3	7.500	7.500	1.196	1.196	
			7.500		1.196		
			7.500		1.196		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	6	F1	8.780	8.780	1.400	1.400	31-Aug-09
			8.780		1.400		
			8.780		1.400		
		F2	8.560	8.560	1.364	1.364	
			8.560		1.364		
			8.560		1.364		
		F3	13.400	13.400	2.136	2.136	
			13.400		2.136		
			13.400		2.136		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	7	F1	10.200	10.200	1.626	1.626	1-Sep-09
			10.200		1.626		
			10.200		1.626		
		F2	9.500	9.500	1.514	1.514	
			9.500		1.514		
			9.500		1.514		
		F3	13.700	13.700	2.184	2.184	
			13.700		2.184		
			13.700		2.184		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	8	F1	8.720	8.720	1.390	1.390	2-Sep-09
			8.720		1.390		
			8.720		1.390		
		F2	8.600	8.600	1.371	1.371	
			8.600		1.371		
			8.600		1.371		
		F3	9.280	9.280	1.479	1.479	
			9.280		1.479		
			9.280		1.479		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	9	F1	11.120	11.120	1.773	1.773	3-Sep-09
			11.120		1.773		
			11.120		1.773		
		F2	12.220	12.220	1.948	1.948	
			12.220		1.948		

			12.220		1.948		
		F3	11.160	11.160	1.779	1.779	
			11.160		1.779		
			11.160		1.779		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N w/out EDTA in STEM	10	F1	9.940	9.940	1.584	1.584	4-Sep-09
			9.940		1.584		
			9.940		1.584		
		F2	12.100	12.100	1.929	1.929	
			12.100		1.929		
			12.100		1.929		
		F3	10.260	10.260	1.635	1.635	
			10.260		1.635		
			10.260		1.635		

Appendix G: Experiment 5 Data

Table G.1: Data for Culture using $NaNO_3$ as "N".

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	0	A1	0.229	0.229	0.037	0.037	11-Sep-09
			0.229		0.037		
			0.229		0.037		
		A2	0.229	0.229	0.037	0.037	
			0.229		0.037		
			0.229		0.037		
		A3	0.229	0.229	0.037	0.037	
			0.229		0.037		
			0.229		0.037		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	1	A1	0.378	0.380	0.060	0.061	12-Sep-09
			0.380		0.061		
			0.382		0.061		
		A2	0.242	0.245	0.039	0.039	
			0.246		0.039		
			0.246		0.039		
		A3	0.460	0.457	0.073	0.073	
			0.452		0.072		
			0.458		0.073		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	2	A1	0.870	0.870	0.139	0.139	13-Sep-09
			0.868		0.138		
			0.872		0.139		
		A2	0.364	0.366	0.058	0.058	
			0.366		0.058		
			0.368		0.059		
		A3	0.934	0.929	0.149	0.148	
			0.922		0.147		
			0.932		0.149		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	3	A1	1.575	1.598	0.251	0.255	14-Sep-09
			1.610		0.257		
			1.610		0.257		
		A2	0.550	0.553	0.088	0.088	
			0.555		0.088		

			0.555		0.088		
		A3	1.825	1.833	0.291	0.292	
			1.840		0.293		
			1.835		0.292		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	4	A1	1.920	1.923	0.306	0.307	15-Sep-09
			1.925		0.307		
			1.925		0.307		
		A2	1.015	1.017	0.162	0.162	
			1.020		0.163		
			1.015		0.162		
		A3	2.510	2.505	0.400	0.399	
			2.500		0.399		
			2.505		0.399		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	5	A1	2.610	2.610	0.416	0.416	16-Sep-09
			2.610		0.416		
			2.610		0.416		
		A2	2.430	2.443	0.387	0.389	
			2.450		0.391		
			2.450		0.391		
		A3	4.030	4.033	0.642	0.643	
			4.050		0.646		
			4.020		0.641		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	6	A1	2.780	2.787	0.443	0.444	17-Sep-09
			2.810		0.448		
			2.770		0.442		
		A2	3.300	3.293	0.526	0.525	
			3.290		0.524		
			3.290		0.524		
		A3	5.030	5.000	0.802	0.797	
			4.990		0.795		
			4.980		0.794		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	7	A1	3.190	3.190	0.508	0.508	18-Sep-09
			3.190		0.508		
			3.190		0.508		
		A2	3.990	3.990	0.636	0.636	
			3.990		0.636		

			3.990		0.636		
		A3	5.700	5.700	0.909	0.909	
			5.700		0.909		
			5.700		0.909		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	8	A1	3.680	3.680	0.587	0.587	19-Sep-09
			3.680		0.587		
			3.680		0.587		
		A2	4.540	4.540	0.724	0.724	
			4.540		0.724		
			4.540		0.724		
		A3	6.300	6.300	1.004	1.004	
			6.300		1.004		
			6.300		1.004		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	9	A1	3.700	3.700	0.590	0.590	20-Sep-09
			3.700		0.590		
			3.700		0.590		
		A2	4.500	4.500	0.717	0.717	
			4.500		0.717		
			4.500		0.717		
		A3	6.400	6.400	1.020	1.020	
			6.400		1.020		
			6.400		1.020		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	10	A1	3.700	3.700	0.590	0.590	21-Sep-09
			3.700		0.590		
			3.700		0.590		
		A2	4.960	4.960	0.791	0.791	
			4.960		0.791		
			4.960		0.791		
		A3	7.460	7.460	1.189	1.189	
			7.460		1.189		
			7.460		1.189		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N ($NaNO_3$)	11	A1	4.280	4.280	0.682	0.682	22-Sep-09
			4.280		0.682		
			4.280		0.682		
		A2	5.600	5.600	0.893	0.893	
			5.600		0.893		

			5.600		0.893		
		A3	5.420	5.420	0.864	0.864	
			5.420		0.864		
			5.420		0.864		

Table G.2: Data for Culture using $(NH_4)_2SO_4$ as "N".

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N $(NH_4)_2SO_4$	0	B1	0.229	0.229	0.037	0.037	11-Sep-09
			0.229		0.037		
			0.229		0.037		
		B2	0.229	0.229	0.037	0.037	
			0.229		0.037		
			0.229		0.037		
		B3	0.229	0.229	0.037	0.037	
			0.229		0.037		
			0.229		0.037		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
BBM4N $(NH_4)_2SO_4$	1	B1	0.368	0.369	0.059	0.059	12-Sep-09
			0.370		0.059		
			0.370		0.059		
		B2	0.390	0.391	0.062	0.062	
			0.390		0.062		
			0.392		0.062		
		B3	0.418	0.413	0.067	0.066	
			0.410		0.065		
			0.412		0.066		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
BBM4N $(NH_4)_2SO_4$	2	B1	0.588	0.589	0.094	0.094	13-Sep-09
			0.590		0.094		
			0.588		0.094		
		B2	1.506	1.501	0.240	0.239	
			1.498		0.239		
			1.500		0.239		
		B3	0.784	0.786	0.125	0.125	
			0.786		0.125		
			0.788		0.126		
BBM4N $(NH_4)_2SO_4$	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	

	3	B1	0.815	0.812	0.130	0.129	14-Sep-09
			0.820		0.131		
			0.800		0.128		
		B2	1.005	1.005	0.160	0.160	
			1.005		0.160		
			1.005		0.160		
		B3	1.210	1.210	0.193	0.193	
			1.210		0.193		
			1.210		0.193		
BBM4N $(NH_4)_2SO_4$	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	4	B1	1.390	1.390	0.222	0.222	15-Sep-09
			1.390		0.222		
			1.390		0.222		
		B2	1.570	1.563	0.250	0.249	
			1.555		0.248		
			1.565		0.249		
		B3	1.595	1.587	0.254	0.253	
			1.590		0.253		
			1.575		0.251		
BBM4N $(NH_4)_2SO_4$	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
	5	B1	2.260	2.243	0.360	0.358	16-Sep-09
			2.240		0.357		
			2.230		0.355		
		B2	2.160	2.147	0.344	0.342	
			2.140		0.341		
			2.140		0.341		
		B3	2.180	2.167	0.347	0.345	
			2.160		0.344		
			2.160		0.344		
BBM4N $(NH_4)_2SO_4$	Day	Culture Vessel	A550	A550 Mean	#VALUE!	Cell Conc. Mean	
	6	B1	2.730	2.770	0.435	0.442	17-Sep-09
			2.810		0.448		
			2.770		0.442		
		B2	2.700	2.717	0.430	0.433	
			2.730		0.435		
			2.720		0.434		
		B3	2.720	2.727	0.434	0.435	
			2.730		0.435		
			2.730		0.435		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
BBM4N $(NH_4)_2SO_4$	7	B1	3.080	3.080	0.491	0.491	18-Sep-09
			3.080		0.491		
			3.080		0.491		
		B2	3.400	3.400	0.542	0.542	
			3.400		0.542		
			3.400		0.542		
		B3	3.260	3.260	0.520	0.520	
			3.260		0.520		
			3.260		0.520		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
BBM4N $(NH_4)_2SO_4$	8	B1	3.960	3.960	0.631	0.631	19-Sep-09
			3.960		0.631		
			3.960		0.631		
		B2	3.680	3.680	0.587	0.587	
			3.680		0.587		
			3.680		0.587		
		B3	3.720	3.720	0.593	0.593	
			3.720		0.593		
			3.720		0.593		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
BBM4N $(NH_4)_2SO_4$	9	B1	3.720	3.720	0.593	0.593	20-Sep-09
			3.720		0.593		
			3.720		0.593		
		B2	3.660	3.660	0.583	0.583	
			3.660		0.583		
			3.660		0.583		
		B3	3.640	3.640	0.580	0.580	
			3.640		0.580		
			3.640		0.580		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
BBM4N $(NH_4)_2SO_4$	10	B1	4.720	4.720	0.752	0.752	21-Sep-09
			4.720		0.752		
			4.720		0.752		
		B2	3.420	3.420	0.545	0.545	
			3.420		0.545		
			3.420		0.545		
		B3	4.280	4.280	0.682	0.682	

			4.280		0.682		
			4.280		0.682		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	
BBM4N $(NH_4)_2SO_4$	11	B1	4.500	4.500	0.717	0.717	22-Sep-09
			4.500		0.717		
			4.500		0.717		
		B2	4.160	4.160	0.663	0.663	
			4.160		0.663		
			4.160		0.663		
		B3	4.360	4.360	0.695	0.695	
			4.360		0.695		
			4.360		0.695		

Table G.3: Data for Culture using NH_4NO_3 as "N".

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	0	C1	0.229	0.229	0.037	0.037	11-Sep-09
			0.229		0.037		
			0.229		0.037		
		C2	0.229	0.229	0.037	0.037	
			0.229		0.037		
			0.229		0.037		
		C3	0.229	0.229	0.037	0.037	
			0.229		0.037		
			0.229		0.037		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	1	C1	0.358	0.359	0.057	0.057	12-Sep-09
			0.362		0.058		
			0.356		0.057		
		C2	0.356	0.355	0.057	0.057	
			0.352		0.056		
			0.356		0.057		
		C3	0.508	0.503	0.081	0.080	
			0.498		0.079		
			0.502		0.080		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	2	C1	0.564	0.566	0.090	0.090	13-Sep-09
			0.566		0.090		

			0.568		0.091		
		C2	0.636	0.637	0.101	0.102	
			0.640		0.102		
			0.636		0.101		
		C3	1.410	1.415	0.225	0.226	
			1.418		0.226		
			1.418		0.226		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	3	C1	0.845	0.842	0.135	0.134	14-Sep-09
			0.845		0.135		
			0.835		0.133		
		C2	0.920	0.925	0.147	0.147	
			0.930		0.148		
			0.925		0.147		
		C3	2.010	2.017	0.320	0.321	
			2.015		0.321		
			2.025		0.323		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	4	C1	1.225	1.228	0.195	0.196	15-Sep-09
			1.230		0.196		
			1.230		0.196		
		C2	1.195	1.198	0.190	0.191	
			1.200		0.191		
			1.200		0.191		
		C3	2.780	2.783	0.443	0.444	
			2.785		0.444		
			2.785		0.444		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	5	C1	2.020	2.020	0.322	0.322	16-Sep-09
			2.030		0.324		
			2.010		0.320		
		C2	1.940	1.947	0.309	0.310	
			1.950		0.311		
			1.950		0.311		
		C3	3.450	3.450	0.550	0.550	
			3.480		0.555		
			3.420		0.545		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	6	C1	2.780	2.810	0.443	0.448	17-Sep-09

			2.830		0.451		
			2.820		0.450		
		C2	2.410	2.410	0.384	0.384	
			2.410		0.384		
			2.410		0.384		
		C3	4.360	4.380	0.695	0.698	
			4.370		0.697		
			4.410		0.703		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	7	C1	3.300	3.300	0.526	0.526	18-Sep-09
			3.300		0.526		
			3.300		0.526		
		C2	2.800	2.800	0.446	0.446	
			2.800		0.446		
			2.800		0.446		
		C3	4.710	4.710	0.751	0.751	
			4.710		0.751		
			4.710		0.751		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	8	C1	3.700	3.700	0.590	0.590	19-Sep-09
			3.700		0.590		
			3.700		0.590		
		C2	3.240	3.240	0.516	0.516	
			3.240		0.516		
			3.240		0.516		
		C3	4.880	4.880	0.778	0.778	
			4.880		0.778		
			4.880		0.778		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	9	C1	4.200	4.200	0.669	0.669	20-Sep-09
			4.200		0.669		
			4.200		0.669		
		C2	3.620	3.620	0.577	0.577	
			3.620		0.577		
			3.620		0.577		
		C3	5.300	5.300	0.845	0.845	
			5.300		0.845		
			5.300		0.845		

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	10	C1	4.000	4.000	0.638	0.638	21-Sep-09
			4.000		0.638		
			4.000		0.638		
		C2	3.260	3.260	0.520	0.520	
			3.260		0.520		
			3.260		0.520		
		C3	4.960	4.960	0.791	0.791	
			4.960		0.791		
			4.960		0.791		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (NH_4NO_3)	11	C1	4.640	4.640	0.740	0.740	22-Sep-09
			4.640		0.740		
			4.640		0.740		
		C2	4.100	4.100	0.654	0.654	
			4.100		0.654		
			4.100		0.654		
		C3	5.080	5.080	0.810	0.810	
			5.080		0.810		
			5.080		0.810		

Table G.4: Data for Culture made as BBM using Commercial Fertilizer & Prilled Urea as “N”.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
CF (BBM) w/ Urea as "N"	0	D1	0.229	0.229	0.037	0.037	11-Sep-09
			0.229		0.037		
			0.229		0.037		
		D2	0.229	0.229	0.037	0.037	
			0.229		0.037		
			0.229		0.037		
		D3	0.229	0.229	0.037	0.037	
			0.229		0.037		
			0.229		0.037		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
CF (BBM) w/ Urea as "N"	1	D1	0.492	0.494	0.078	0.079	12-Sep-09
			0.496		0.079		
			0.494		0.079		
		D2	0.414	0.413	0.066	0.066	

			0.412		0.066		
			0.414		0.066		
		D3	0.498		0.079		
			0.496	0.496	0.079	0.079	
			0.494		0.079		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		D1	1.054		0.168		
			1.060	1.057	0.169	0.169	
			1.058		0.169		
CF (BBM) w/ Urea as "N"	2	D2	0.626		0.100		
			0.630	0.627	0.100	0.100	13-Sep-09
			0.626		0.100		
		D3	1.144		0.182		
			1.146	1.144	0.183	0.182	
			1.142		0.182		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		D1	1.615		0.257		
			1.625	1.623	0.259	0.259	
			1.630		0.260		
CF (BBM) w/ Urea as "N"	3	D2	0.875		0.139		
			0.875	0.875	0.139	0.139	14-Sep-09
			0.875		0.139		
		D3	1.750		0.279		
			1.765	1.757	0.281	0.280	
			1.755		0.280		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		D1	2.310		0.368		
			2.305	2.307	0.367	0.368	
			2.305		0.367		
CF (BBM) w/ Urea as "N"	4	D2	1.215		0.194		
			1.210	1.212	0.193	0.193	15-Sep-09
			1.210		0.193		
		D3	2.455		0.391		
			2.460	2.458	0.392	0.392	
			2.460		0.392		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
CF (BBM) w/ Urea as "N"	5	D1	2.910		0.464		
			2.920	2.917	0.465	0.465	16-Sep-09

			2.920		0.465		
		D2	2.060	2.060	0.328	0.328	
			2.060		0.328		
			2.060		0.328		
		D3	3.090	3.103	0.493	0.495	
			3.110		0.496		
			3.110		0.496		
CF (BBM) w/ Urea as "N"	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	D1	3.480	3.527	0.555	0.562	17-Sep-09
			3.570		0.569		
			3.530		0.563		
		D2	2.650	2.650	0.422	0.422	
			2.650		0.422		
			2.650		0.422		
		D3	3.900	3.900	0.622	0.622	
			3.900		0.622		
			3.900		0.622		
CF (BBM) w/ Urea as "N"	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	7	D1	4.030	4.030	0.642	0.642	18-Sep-09
			4.030		0.642		
			4.030		0.642		
		D2	3.090	3.090	0.493	0.493	
			3.090		0.493		
			3.090		0.493		
		D3	4.370	4.370	0.697	0.697	
			4.370		0.697		
			4.370		0.697		
BBM4N (Urea)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	D1	3.960	3.960	0.631	0.631	19-Sep-09
			3.960		0.631		
			3.960		0.631		
		D2	3.640	3.640	0.580	0.580	
			3.640		0.580		
			3.640		0.580		
		D3	5.340	5.340	0.851	0.851	
			5.340		0.851		
			5.340		0.851		
BBM4N (Urea)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date

	9	D1	4.000	4.000	0.638	0.638	20-Sep-09
			4.000		0.638		
			4.000		0.638		
		D2	3.640	3.640	0.580	0.580	
			3.640		0.580		
			3.640		0.580		
		D3	5.400	5.400	0.861	0.861	
			5.400		0.861		
			5.400		0.861		
BBM4N (Urea)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	10	D1	4.540	4.540	0.724	0.724	21-Sep-09
			4.540		0.724		
			4.540		0.724		
		D2	3.880	3.880	0.618	0.618	
			3.880		0.618		
			3.880		0.618		
		D3	5.300	5.300	0.845	0.845	
			5.300		0.845		
			5.300		0.845		
BBM4N (Urea)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	11	D1	5.000	5.000	0.797	0.797	22-Sep-09
			5.000		0.797		
			5.000		0.797		
		D2	4.380	4.380	0.698	0.698	
			4.380		0.698		
			4.380		0.698		
		D3	6.680	6.680	1.065	1.065	
			6.680		1.065		
			6.680		1.065		

Table G.5: Data for Culture made as BBM using Commercial Fertilizer & Autoclave.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (CF w/ autoclave, KNO3 as "N")	0	E1	0.148	0.148	0.024	0.024	11-Sep-09
			0.148		0.024		
			0.148		0.024		
		E2	0.148	0.148	0.024	0.024	
			0.148		0.024		
			0.148		0.024		

		E3	0.148	0.148	0.024	0.024	
			0.148		0.024		
			0.148		0.024		
BBM4N (CF w/ autoclave, KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	1	E1	0.274	0.277	0.044	0.044	12-Sep-09
			0.276		0.044		
			0.280		0.045		
		E2	0.372	0.369	0.059	0.059	
			0.366		0.058		
			0.370		0.059		
		E3	0.332	0.332	0.053	0.053	
			0.330		0.053		
			0.334		0.053		
BBM4N (CF w/ autoclave, KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	2	E1	0.606	0.609	0.097	0.097	13-Sep-09
			0.610		0.097		
			0.612		0.098		
		E2	1.046	1.051	0.167	0.168	
			1.060		0.169		
			1.048		0.167		
		E3	0.960	0.961	0.153	0.153	
			0.962		0.153		
			0.960		0.153		
BBM4N (CF w/ autoclave, KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	3	E1	0.860	0.860	0.137	0.137	14-Sep-09
			0.860		0.137		
			0.860		0.137		
		E2	1.785	1.788	0.285	0.285	
			1.790		0.285		
			1.790		0.285		
		E3	1.590	1.587	0.253	0.253	
			1.585		0.253		
			1.585		0.253		
BBM4N (CF w/ autoclave, KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	E1	2.820	2.830	0.450	0.451	15-Sep-09
			2.830		0.451		
			2.840		0.453		
		E2	2.610	2.610	0.416	0.416	
			2.610		0.416		
			2.610		0.416		

		E3	2.445	2.442	0.390	0.389	
			2.440		0.389		
			2.440		0.389		
BBM4N (CF w/ autoclave, KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	5	E1	1.940	1.953	0.309	0.311	16-Sep-09
			1.960		0.312		
			1.960		0.312		
		E2	3.440	3.440	0.548	0.548	
			3.440		0.548		
			3.440		0.548		
		E3	3.210	3.210	0.512	0.512	
			3.210		0.512		
			3.210		0.512		
BBM4N (CF w/ autoclave, KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	E1	2.630	2.603	0.419	0.415	17-Sep-09
			2.590		0.413		
			2.590		0.413		
		E2	3.920	3.937	0.625	0.628	
			3.950		0.630		
			3.940		0.628		
		E3	4.330	4.313	0.690	0.688	
			4.300		0.685		
			4.310		0.687		
BBM4N (CF w/ autoclave, KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	7	E1	2.990	2.990	0.477	0.477	18-Sep-09
			2.990		0.477		
			2.990		0.477		
		E2	4.560	4.560	0.727	0.727	
			4.560		0.727		
			4.560		0.727		
		E3	5.280	5.280	0.842	0.842	
			5.280		0.842		
			5.280		0.842		
BBM4N (CF w/ EDTA STEM)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	E1	3.640	3.640	0.580	0.580	19-Sep-09
			3.640		0.580		
			3.640		0.580		
		E2	4.980	4.980	0.794	0.794	
			4.980		0.794		
			4.980		0.794		

		E3	5.880	5.880	0.937	0.937	
			5.880		0.937		
			5.880		0.937		
BBM4N (CF w/ EDTA STEM)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	E1	3.440	3.440	0.548	0.548	20-Sep-09
			3.440		0.548		
			3.440		0.548		
		E2	5.260	5.260	0.838	0.838	
			5.260		0.838		
			5.260		0.838		
		E3	6.220	6.220	0.991	0.991	
			6.220		0.991		
			6.220		0.991		
BBM4N (CF w/ EDTA STEM)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	10	E1	3.380	3.380	0.539	0.539	21-Sep-09
			3.380		0.539		
			3.380		0.539		
		E2	4.760	4.760	0.759	0.759	
			4.760		0.759		
			4.760		0.759		
		E3	5.860	5.860	0.934	0.934	
			5.860		0.934		
			5.860		0.934		
BBM4N (CF w/ EDTA STEM)	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	11	E1	4.160	4.160	0.663	0.663	22-Sep-09
			4.160		0.663		
			4.160		0.663		
		E2	5.760	5.760	0.918	0.918	
			5.760		0.918		
			5.760		0.918		
		E3	7.400	7.400	1.180	1.180	
			7.400		1.180		
			7.400		1.180		

Table G.6: Data for Culture made as BBM using Commercial Fertilizer & No Autoclave.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (w/out autoclave, w/ KNO3 as "N")	0	F1	0.148	0.148	0.024	0.024	11-Sep-09
			0.148		0.024		
			0.148		0.024		
		F2	0.148	0.148	0.024	0.024	
			0.148		0.024		
			0.148		0.024		
		F3	0.148	0.148	0.024	0.024	
			0.148		0.024		
			0.148		0.024		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (w/out autoclave, w/ KNO3 as "N")	1	F1	0.360	0.357	0.057	0.057	12-Sep-09
			0.352		0.056		
			0.360		0.057		
		F2	0.362	0.364	0.058	0.058	
			0.366		0.058		
			0.364		0.058		
		F3	0.380	0.379	0.061	0.060	
			0.376		0.060		
			0.380		0.061		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (w/out autoclave, w/ KNO3 as "N")	2	F1	0.784	0.785	0.125	0.125	13-Sep-09
			0.786		0.125		
			0.784		0.125		
		F2	0.770	0.771	0.123	0.123	
			0.772		0.123		
			0.772		0.123		
		F3	0.938	0.937	0.150	0.149	
			0.936		0.149		
			0.936		0.149		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (w/out autoclave, w/ KNO3 as "N")	3	F1	1.140	1.140	0.182	0.182	14-Sep-09
			1.135		0.181		
			1.145		0.183		
		F2	0.895	0.892	0.143	0.142	
			0.890		0.142		
			0.890		0.142		

		F3	1.380	1.380	0.220	0.220	
			1.380		0.220		
			1.380		0.220		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (w/out autoclave, w/ KNO3 as "N")	4	F1	1.655	1.653	0.264	0.264	15-Sep-09
			1.655		0.264		
			1.650		0.263		
		F2	1.145	1.143	0.183	0.182	
			1.145		0.183		
			1.140		0.182		
		F3	1.825	1.828	0.291	0.291	
			1.830		0.292		
			1.830		0.292		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (w/out autoclave, w/ KNO3 as "N")	5	F1	2.250	2.250	0.359	0.359	16-Sep-09
			2.250		0.359		
			2.250		0.359		
		F2	1.700	1.700	0.271	0.271	
			1.700		0.271		
			1.700		0.271		
		F3	2.530	2.540	0.403	0.405	
			2.540		0.405		
			2.550		0.406		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (w/out autoclave, w/ KNO3 as "N")	6	F1	3.010	3.023	0.480	0.482	17-Sep-09
			3.020		0.481		
			3.040		0.485		
		F2	2.460	2.460	0.392	0.392	
			2.460		0.392		
			2.460		0.392		
		F3	3.270	3.280	0.521	0.523	
			3.280		0.523		
			3.290		0.524		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
BBM4N (w/out autoclave, w/ KNO3 as "N")	7	F1	3.610	3.610	0.575	0.575	18-Sep-09
			3.610		0.575		
			3.610		0.575		
		F2	3.150	3.150	0.502	0.502	
			3.150		0.502		
			3.150		0.502		

		F3	3.640	3.640	0.580	0.580	
			3.640		0.580		
			3.640		0.580		
BBM4N (w/out autoclave, w/ KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	F1	4.020	4.020	0.641	0.641	19-Sep-09
			4.020		0.641		
			4.020		0.641		
		F2	3.640	3.640	0.580	0.580	
			3.640		0.580		
			3.640		0.580		
		F3	4.040	4.040	0.644	0.644	
			4.040		0.644		
			4.040		0.644		
BBM4N (w/out autoclave, w/ KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	F1	4.040	4.040	0.644	0.644	20-Sep-09
			4.040		0.644		
			4.040		0.644		
		F2	3.240	3.240	0.516	0.516	
			3.240		0.516		
			3.240		0.516		
		F3	4.300	4.300	0.685	0.685	
			4.300		0.685		
			4.300		0.685		
BBM4N (w/out autoclave, w/ KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	10	F1	3.880	3.880	0.618	0.618	21-Sep-09
			3.880		0.618		
			3.880		0.618		
		F2	3.220	3.220	0.513	0.513	
			3.220		0.513		
			3.220		0.513		
		F3	4.480	4.480	0.714	0.714	
			4.480		0.714		
			4.480		0.714		
BBM4N (w/out autoclave, w/ KNO3 as "N")	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	11	F1	4.220	4.220	0.673	0.673	22-Sep-09
			4.220		0.673		
			4.220		0.673		
		F2	3.800	3.800	0.606	0.606	
			3.800		0.606		
			3.800		0.606		

			5.000		0.797		
		F3	5.000	5.000	0.797	0.797	
			5.000		0.797		

Appendix H: Experiment 6 Data

Table H.1: Data for Culture exposed to a 24:0 Light / Dark Photoperiod.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
24:0	0	A1	0.194	0.194	0.031	0.031	1-Oct-09
			0.194		0.031		
			0.194		0.031		
		A2	0.191	0.191	0.030	0.030	
			0.191		0.030		
			0.191		0.030		
		A3	0.196	0.196	0.031	0.031	
			0.196		0.031		
			0.196		0.031		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
24:0	1	A1	0.140	0.140	0.022	0.022	2-Oct-09
			0.140		0.022		
			0.140		0.022		
		A2	0.183	0.183	0.029	0.029	
			0.183		0.029		
			0.183		0.029		
		A3	0.150	0.150	0.024	0.024	
			0.150		0.024		
			0.150		0.024		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
24:0	2	A1	0.127	0.127	0.020	0.020	3-Oct-09
			0.127		0.020		
			0.127		0.020		
		A2	0.105	0.105	0.017	0.017	
			0.105		0.017		
			0.105		0.017		
		A3	0.111	0.111	0.018	0.018	
			0.111		0.018		
			0.111		0.018		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
24:0	3	A1	0.146	0.146	0.023	0.023	4-Oct-09
			0.146		0.023		
			0.146		0.023		
		A2	0.198	0.198	0.032	0.032	

			0.198		0.032		
			0.198		0.032		
		A3	0.293	0.293	0.047	0.047	
			0.293		0.047		
			0.293		0.047		
24:0	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	A1	0.196	0.196	0.031	0.031	5-Oct-09
			0.196		0.031		
			0.196		0.031		
		A2	0.439	0.439	0.070	0.070	
			0.439		0.070		
			0.439		0.070		
		A3	0.670	0.670	0.107	0.107	
			0.670		0.107		
			0.670		0.107		
24:0	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	5	A1	0.420	0.419	0.067	0.067	6-Oct-09
			0.418		0.067		
			0.418		0.067		
		A2	0.976	0.980	0.156	0.156	
			0.984		0.157		
			0.979		0.156		
		A3	1.320	1.324	0.210	0.211	
			1.328		0.212		
			1.323		0.211		
24:0	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	A1	0.662	0.654	0.106	0.104	7-Oct-09
			0.648		0.103		
			0.652		0.104		
		A2	1.794	1.789	0.286	0.285	
			1.784		0.284		
			1.788		0.285		
		A3	2.318	2.310	0.369	0.368	
			2.302		0.367		
			2.310		0.368		
24:0	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	7	A1	1.700	1.703	0.271	0.272	8-Oct-09
			1.700		0.271		
			1.710		0.273		
		A2	3.420	3.423	0.545	0.546	

			3.420		0.545		
			3.430		0.547		
		A3	4.400	4.413	0.701	0.703	
			4.420		0.705		
			4.420		0.705		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	A1	3.270	3.230	0.521	0.515	9-Oct-09
			3.190		0.508		
			3.230		0.515		
24:0		A2	4.380	4.363	0.698	0.696	
			4.350		0.693		
			4.360		0.695		
		A3	5.660	5.703	0.902	0.909	
			5.760		0.918		
			5.690		0.907		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	A1	3.950	3.970	0.630	0.633	10-Oct-09
			3.980		0.634		
			3.980		0.634		
24:0		A2	5.170	5.187	0.824	0.827	
			5.200		0.829		
			5.190		0.827		
		A3	6.840	6.817	1.090	1.087	
			6.800		1.084		
			6.810		1.086		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	10	A1	5.410	5.423	0.862	0.864	11-Oct-09
			5.430		0.866		
			5.430		0.866		
24:0		A2	6.340	6.353	1.011	1.013	
			6.360		1.014		
			6.360		1.014		
		A3	7.810	7.810	1.245	1.245	
			7.810		1.245		
			7.810		1.245		

Table H.2: Data for Culture exposed to an 18:6 Light / Dark Photoperiod.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
18:6	0	B1	0.196	0.196	0.031	0.031	1-Oct-09
			0.196		0.031		
			0.196		0.031		
		B2	0.201	0.201	0.032	0.032	
			0.201		0.032		
			0.201		0.032		
		B3	0.202	0.202	0.032	0.032	
			0.202		0.032		
			0.202		0.032		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
18:6	1	B1	0.141	0.141	0.022	0.022	2-Oct-09
			0.141		0.022		
			0.141		0.022		
		B2	0.152	0.152	0.024	0.024	
			0.152		0.024		
			0.152		0.024		
		B3	0.151	0.151	0.024	0.024	
			0.151		0.024		
			0.151		0.024		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
18:6	2	B1	0.100	0.100	0.016	0.016	3-Oct-09
			0.100		0.016		
			0.100		0.016		
		B2	0.110	0.110	0.018	0.018	
			0.110		0.018		
			0.110		0.018		
		B3	0.137	0.137	0.022	0.022	
			0.137		0.022		
			0.137		0.022		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
18:6	3	B1	0.197	0.197	0.031	0.031	4-Oct-09
			0.197		0.031		
			0.197		0.031		
		B2	0.153	0.153	0.024	0.024	
			0.153		0.024		
			0.153		0.024		

		B3	0.141	0.141	0.022	0.022	
			0.141		0.022		
			0.141		0.022		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
18:6	4	B1	0.325	0.325	0.052	0.052	5-Oct-09
			0.325		0.052		
			0.325		0.052		
		B2	0.240	0.240	0.038	0.038	
			0.240		0.038		
			0.240		0.038		
		B3	0.163	0.163	0.026	0.026	
			0.163		0.026		
			0.163		0.026		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
18:6	5	B1	0.681	0.687	0.109	0.110	6-Oct-09
			0.690		0.110		
			0.690		0.110		
		B2	0.910	0.911	0.145	0.145	
			0.910		0.145		
			0.912		0.145		
		B3	0.320	0.319	0.051	0.051	
			0.319		0.051		
			0.319		0.051		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
18:6	6	B1	1.236	1.236	0.197	0.197	7-Oct-09
			1.236		0.197		
			1.236		0.197		
		B2	0.704	0.703	0.112	0.112	
			0.702		0.112		
			0.702		0.112		
		B3	0.570	0.565	0.091	0.090	
			0.562		0.090		
			0.564		0.090		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
18:6	7	B1	2.505	2.500	0.399	0.399	8-Oct-09
			2.495		0.398		
			2.500		0.399		
		B2	1.310	1.310	0.209	0.209	
			1.310		0.209		

			1.310		0.209		
		B3	1.525	1.522	0.243	0.243	
			1.520		0.242		
			1.520		0.242		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	B1	3.850	3.810	0.614	0.607	9-Oct-09
			3.780		0.603		
			3.800		0.606		
18:6		B2	2.080	2.060	0.332	0.328	
			2.040		0.325		
			2.060		0.328		
		B3	2.950	2.960	0.470	0.472	
			2.960		0.472		
			2.970		0.473		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	B1	4.720	4.737	0.752	0.755	10-Oct-09
			4.730		0.754		
			4.760		0.759		
18:6		B2	2.460	2.497	0.392	0.398	
			2.520		0.402		
			2.510		0.400		
		B3	3.880	3.860	0.618	0.615	
			3.850		0.614		
			3.850		0.614		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	10	B1	5.650	5.637	0.901	0.898	11-Oct-09
			5.630		0.897		
			5.630		0.897		
18:6		B2	3.170	3.183	0.505	0.507	
			3.190		0.508		
			3.190		0.508		
		B3	5.350	5.303	0.853	0.845	
			5.270		0.840		
			5.290		0.843		

Table H.3: Data for Culture exposed to a 12:12 Light / Dark Photoperiod.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
12:12	0	C1	0.204	0.204	0.033	0.033	1-Oct-09
			0.204		0.033		
			0.204		0.033		
		C2	0.197	0.197	0.031	0.031	
			0.197		0.031		
			0.197		0.031		
		C3	0.208	0.208	0.033	0.033	
			0.208		0.033		
			0.208		0.033		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
12:12	1	C1	0.137	0.137	0.022	0.022	2-Oct-09
			0.137		0.022		
			0.137		0.022		
		C2	0.154	0.154	0.025	0.025	
			0.154		0.025		
			0.154		0.025		
		C3	0.140	0.140	0.022	0.022	
			0.140		0.022		
			0.140		0.022		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
12:12	2	C1	0.107	0.107	0.017	0.017	3-Oct-09
			0.107		0.017		
			0.107		0.017		
		C2	0.112	0.112	0.018	0.018	
			0.112		0.018		
			0.112		0.018		
		C3	0.113	0.113	0.018	0.018	
			0.113		0.018		
			0.113		0.018		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
12:12	3	C1	0.099	0.099	0.016	0.016	4-Oct-09
			0.099		0.016		
			0.099		0.016		
		C2	0.103	0.103	0.016	0.016	
			0.103		0.016		
			0.103		0.016		

		C3	0.109	0.109	0.017	0.017	
			0.109		0.017		
			0.109		0.017		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
12:12	4	C1	0.088	0.088	0.014	0.014	5-Oct-09
			0.088		0.014		
			0.088		0.014		
		C2	0.089	0.089	0.014	0.014	
			0.089		0.014		
			0.089		0.014		
		C3	0.103	0.103	0.016	0.016	
			0.103		0.016		
			0.103		0.016		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
12:12	5	C1	0.095	0.096	0.015	0.015	6-Oct-09
			0.097		0.015		
			0.097		0.015		
		C2	0.098	0.096	0.016	0.015	
			0.094		0.015		
			0.096		0.015		
		C3	0.113	0.115	0.018	0.018	
			0.117		0.019		
			0.116		0.018		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
12:12	6	C1	0.098	0.099	0.016	0.016	7-Oct-09
			0.101		0.016		
			0.099		0.016		
		C2	0.092	0.091	0.015	0.015	
			0.090		0.014		
			0.091		0.015		
		C3	0.105	0.105	0.017	0.017	
			0.104		0.017		
			0.105		0.017		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
12:12	7	C1	0.109	0.111	0.017	0.018	8-Oct-09
			0.112		0.018		
			0.113		0.018		
		C2	0.085	0.087	0.014	0.014	
			0.087		0.014		

			0.088		0.014		
		C3	0.106	0.106	0.017	0.017	
			0.106		0.017		
			0.106		0.017		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	C1	0.111	0.112	0.018	0.018	
			0.113		0.018		
			0.112		0.018		
12:12		C2	0.102	0.102	0.016	0.016	9-Oct-09
			0.102		0.016		
			0.103		0.016		
		C3	0.097	0.102	0.015	0.016	
			0.107		0.017		
			0.103		0.016		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	C1	0.103	0.104	0.016	0.017	
			0.104		0.017		
			0.105		0.017		
12:12		C2	0.125	0.125	0.020	0.020	10-Oct-09
			0.124		0.020		
			0.126		0.020		
		C3	0.087	0.089	0.014	0.014	
			0.089		0.014		
			0.090		0.014		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	11	C1	0.106	0.105	0.017	0.017	
			0.107		0.017		
			0.103		0.016		
12:12		C2	0.215	0.210	0.034	0.034	8-Oct-09
			0.207		0.033		
			0.209		0.033		
		C3	0.078	0.081	0.012	0.013	
			0.083		0.013		
			0.082		0.013		

Table H.4: Data for Culture exposed to a 6:12 Light / Dark Photoperiod.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
6:18	0	D1	0.191	0.191	0.030	0.030	1-Oct-09
			0.191		0.030		
			0.191		0.030		
		D2	0.200	0.200	0.032	0.032	
			0.200		0.032		
			0.200		0.032		
		D3	0.197	0.197	0.031	0.031	
			0.197		0.031		
			0.197		0.031		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
6:18	1	D1	0.141	0.141	0.022	0.022	2-Oct-09
			0.141		0.022		
			0.141		0.022		
		D2	0.157	0.157	0.025	0.025	
			0.157		0.025		
			0.157		0.025		
		D3	0.136	0.136	0.022	0.022	
			0.136		0.022		
			0.136		0.022		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
6:18	2	D1	0.116	0.116	0.018	0.018	3-Oct-09
			0.116		0.018		
			0.116		0.018		
		D2	0.124	0.124	0.020	0.020	
			0.124		0.020		
			0.124		0.020		
		D3	0.094	0.094	0.015	0.015	
			0.094		0.015		
			0.094		0.015		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
6:18	3	D1	0.099	0.099	0.016	0.016	4-Oct-09
			0.099		0.016		
			0.099		0.016		
		D2	0.113	0.113	0.018	0.018	
			0.113		0.018		

			0.113		0.018		
		D3	0.088	0.088	0.014	0.014	
			0.088		0.014		
			0.088		0.014		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		D1	0.067	0.067	0.011	0.011	
			0.067		0.011		
			0.067		0.011		
6:18	4	D2	0.088	0.088	0.014	0.014	5-Oct-09
			0.088		0.014		
			0.088		0.014		
		D3	0.076	0.076	0.012	0.012	
			0.076		0.012		
			0.076		0.012		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		D1	0.073	0.075	0.012	0.012	
			0.076		0.012		
			0.077		0.012		
6:18	5	D2	0.090	0.091	0.014	0.014	6-Oct-09
			0.091		0.015		
			0.091		0.015		
		D3	0.067	0.069	0.011	0.011	
			0.070		0.011		
			0.071		0.011		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		D1	0.064	0.065	0.010	0.010	
			0.065		0.010		
			0.065		0.010		
6:18	6	D2	0.087	0.087	0.014	0.014	7-Oct-09
			0.087		0.014		
			0.088		0.014		
		D3	0.062	0.064	0.010	0.010	
			0.065		0.010		
			0.064		0.010		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		D1	0.072	0.067	0.011	0.011	
6:18	7		0.062		0.010		8-Oct-09
			0.066		0.011		
		D2	0.089	0.089	0.014	0.014	

			0.088		0.014		
			0.089		0.014		
		D3	0.070		0.011		
			0.070	0.070	0.011	0.011	
			0.070		0.011		
6:18	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	D1	0.072		0.011		
			0.072	0.072	0.011	0.012	
			0.073		0.012		
		D2	0.095		0.015		
			0.094	0.094	0.015	0.015	9-Oct-09
			0.093		0.015		
		D3	0.066		0.011		
			0.070	0.068	0.011	0.011	
			0.068		0.011		
6:18	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	D1	0.059		0.009		
			0.061	0.060	0.010	0.010	
			0.061		0.010		
		D2	0.073		0.012		
			0.075	0.074	0.012	0.012	10-Oct-09
			0.075		0.012		
		D3	0.072		0.011		
			0.070	0.071	0.011	0.011	
			0.071		0.011		
6:18	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	10	D1	0.069		0.011		
			0.071	0.070	0.011	0.011	
			0.070		0.011		
		D2	0.089		0.014		
			0.089	0.089	0.014	0.014	11-Oct-09
			0.090		0.014		
		D3	0.086		0.014		
			0.085	0.085	0.014	0.014	
			0.084		0.013		

Appendix I: Experiment 7 Data

Table I.1: Data for Culture Grown on 4% CO_2.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
4%CO_2	0	A1	0.239	0.240	0.039	0.039	14-Oct-09
			0.240		0.039		
			0.241		0.039		
		A2	0.269	0.270	0.043	0.044	
			0.270		0.044		
			0.271		0.044		
		A3	0.227	0.227	0.037	0.037	
			0.227		0.037		
			0.227		0.037		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
4%CO_2	1	A1	0.451	0.449	0.073	0.072	15-Oct-09
			0.447		0.072		
			0.448		0.072		
		A2	0.467	0.469	0.075	0.076	
			0.470		0.076		
			0.469		0.076		
		A3	0.328	0.326	0.053	0.053	
			0.325		0.052		
			0.326		0.053		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
4%CO_2	2	A1	0.778	0.779	0.125	0.126	16-Oct-09
			0.780		0.126		
			0.779		0.126		
		A2	0.754	0.753	0.122	0.121	
			0.751		0.121		
			0.753		0.121		
		A3	0.519	0.519	0.084	0.084	
			0.519		0.084		
			0.519		0.084		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
4%CO_2	3	A1	1.980	1.970	0.319	0.318	17-Oct-09
			1.970		0.318		
			1.960		0.316		
		A2	2.030	2.050	0.327	0.331	
			2.070		0.334		

			2.050		0.331		
		A3	1.580	1.567	0.255	0.253	
			1.550		0.250		
			1.570		0.253		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
4%CO_2	4	A1	3.460	3.477	0.558	0.561	18-Oct-09
			3.490		0.563		
			3.480		0.561		
		A2	3.520	3.537	0.568	0.570	
			3.550		0.573		
			3.540		0.571		
		A3	2.730	2.753	0.440	0.444	
			2.760		0.445		
			2.770		0.447		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
4%CO_2	5	A1	5.100	5.123	0.823	0.826	19-Oct-09
			5.150		0.831		
			5.120		0.826		
		A2	5.530	5.523	0.892	0.891	
			5.520		0.890		
			5.520		0.890		
		A3	3.840	3.850	0.619	0.621	
			3.850		0.621		
			3.860		0.623		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
4%CO_2	6	A1	6.290	6.320	1.015	1.019	20-Oct-09
			6.340		1.023		
			6.330		1.021		
		A2	6.770	6.790	1.092	1.095	
			6.790		1.095		
			6.810		1.098		
		A3	5.510	5.517	0.889	0.890	
			5.510		0.889		
			5.530		0.892		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
4%CO_2	7	A1	7.310	7.310	1.179	1.179	21-Oct-09
			7.310		1.179		
			7.310		1.179		
		A2	8.320	8.350	1.342	1.347	
			8.370		1.350		

			8.360		1.348		
		A3	7.580	7.607	1.223	1.227	
			7.630		1.231		
			7.610		1.227		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
$4\%CO_2$	8	A1	8.330	8.353	1.344	1.347	22-Oct-09
			8.370		1.350		
			8.360		1.348		
		A2	9.580	9.563	1.545	1.543	
			9.550		1.540		
			9.560		1.542		
		A3	9.300	9.300	1.500	1.500	
			9.300		1.500		
			9.300		1.500		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
$4\%CO_2$	9	A1	12.620	12.580	2.036	2.029	23-Oct-09
			12.580		2.029		
			12.540		2.023		
		A2	13.240	13.240	2.136	2.136	
			13.240		2.136		
			13.240		2.136		
		A3	13.300	13.287	2.145	2.143	
			13.280		2.142		
			13.280		2.142		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
$4\%CO_2$	10	A1	14.480	14.473	2.336	2.335	24-Oct-09
			14.480		2.336		
			14.460		2.332		
		A2	14.580	14.640	2.352	2.361	
			14.660		2.365		
			14.680		2.368		
		A3	13.960	13.860	2.252	2.236	
			13.780		2.223		
			13.840		2.232		

Table I.2: Data for Culture Grown on Ambient Air (.04%) CO_2.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	0	B1	0.240	0.240	0.039	0.039	14-Oct-09
			0.239		0.039		
			0.241		0.039		
		B2	0.260	0.259	0.042	0.042	
			0.258		0.042		
			0.258		0.042		
		B3	0.257	0.258	0.041	0.042	
			0.258		0.042		
			0.259		0.042		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	1	B1	0.385	0.388	0.062	0.063	15-Oct-09
			0.389		0.063		
			0.390		0.063		
		B2	0.359	0.357	0.058	0.058	
			0.354		0.057		
			0.357		0.058		
		B3	0.352	0.353	0.057	0.057	
			0.354		0.057		
			0.354		0.057		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	2	B1	0.746	0.747	0.120	0.120	16-Oct-09
			0.747		0.120		
			0.747		0.120		
		B2	0.756	0.756	0.122	0.122	
			0.755		0.122		
			0.756		0.122		
		B3	0.789	0.787	0.127	0.127	
			0.787		0.127		
			0.786		0.127		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	3	B1	1.940	1.957	0.313	0.316	17-Oct-09
			1.970		0.318		
			1.960		0.316		
		B2	1.820	1.833	0.294	0.296	
			1.850		0.298		
			1.830		0.295		
		B3	2.000	1.993	0.323	0.322	

			1.990		0.321		
			1.990		0.321		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	4	B1	2.750	2.723	0.444	0.439	18-Oct-09
			2.690		0.434		
			2.730		0.440		
		B2	2.550	2.550	0.411	0.411	
			2.540		0.410		
			2.560		0.413		
		B3	2.830	2.820	0.456	0.455	
			2.820		0.455		
			2.810		0.453		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	5	B1	3.520	3.523	0.568	0.568	19-Oct-09
			3.520		0.568		
			3.530		0.569		
		B2	3.140	3.127	0.506	0.504	
			3.120		0.503		
			3.120		0.503		
		B3	3.230	3.253	0.521	0.525	
			3.260		0.526		
			3.270		0.527		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	6	B1	4.640	4.663	0.748	0.752	20-Oct-09
			4.680		0.755		
			4.670		0.753		
		B2	3.730	3.730	0.602	0.602	
			3.730		0.602		
			3.730		0.602		
		B3	3.900	3.923	0.629	0.633	
			3.930		0.634		
			3.940		0.636		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	7	B1	5.780	5.800	0.932	0.936	21-Oct-09
			5.830		0.940		
			5.790		0.934		
		B2	5.040	5.003	0.813	0.807	
			4.980		0.803		
			4.990		0.805		
		B3	4.640	4.633	0.748	0.747	

			4.630		0.747		
			4.630		0.747		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	8	B1	6.670	6.663	1.076	1.075	22-Oct-09
			6.680		1.077		
			6.640		1.071		
		B2	5.790	5.807	0.934	0.937	
			5.820		0.939		
			5.810		0.937		
		B3	5.440	5.427	0.877	0.875	
			5.420		0.874		
			5.420		0.874		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	9	B1	9.080	9.087	1.465	1.466	23-Oct-09
			9.080		1.465		
			9.100		1.468		
		B2	8.200	8.233	1.323	1.328	
			8.240		1.329		
			8.260		1.332		
		B3	7.160	7.127	1.155	1.150	
			7.080		1.142		
			7.140		1.152		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
Ambient	10	B1	11.260	11.227	1.816	1.811	24-Oct-09
			11.220		1.810		
			11.200		1.807		
		B2	9.200	9.133	1.484	1.473	
			9.080		1.465		
			9.120		1.471		
		B3	6.900	6.860	1.113	1.107	
			6.760		1.090		
			6.920		1.116		

Table I.3: Data for Culture Grown on 10% CO_2.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
10% CO_2	0	A1	0.252	0.251	0.041	0.040	26-Oct-09
			0.251		0.040		
			0.250		0.040		
		A2	0.255	0.256	0.041	0.041	

			0.255		0.041		
			0.257		0.041		
		A3	0.240	0.241	0.039	0.039	
			0.241		0.039		
			0.241		0.039		
10% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	1	A1	0.470	0.472	0.076	0.076	27-Oct-09
			0.473		0.076		
			0.472		0.076		
		A2	0.517	0.516	0.083	0.083	
			0.514		0.083		
			0.516		0.083		
		A3	0.518	0.518	0.084	0.084	
			0.518		0.084		
			0.518		0.084		
10% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	2	A1	0.633	0.632	0.102	0.102	28-Oct-09
			0.631		0.102		
			0.631		0.102		
		A2	0.658	0.656	0.106	0.106	
			0.653		0.105		
			0.656		0.106		
		A3	0.721	0.723	0.116	0.117	
			0.723		0.117		
			0.725		0.117		
10% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	3	A1	1.960	1.967	0.316	0.317	29-Oct-09
			1.970		0.318		
			1.970		0.318		
		A2	2.040	2.033	0.329	0.328	
			2.040		0.329		
			2.020		0.326		
		A3	1.970	1.970	0.318	0.318	
			1.970		0.318		
			1.970		0.318		
10% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	A1	2.460	2.467	0.397	0.398	30-Oct-09
			2.460		0.397		
			2.480		0.400		
		A2	2.580	2.590	0.416	0.418	

			2.600		0.419		
			2.590		0.418		
		A3	2.660	2.657	0.429	0.429	
			2.660		0.429		
			2.650		0.427		
10% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	5	A1	5.600	5.607	0.903	0.904	31-Oct-09
			5.600		0.903		
			5.620		0.907		
		A2	4.280	4.260	0.690	0.687	
			4.260		0.687		
			4.240		0.684		
		A3	5.660	5.653	0.913	0.912	
			5.620		0.907		
			5.680		0.916		
10% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	A1	6.060	6.067	0.977	0.979	1-Nov-09
			6.060		0.977		
			6.080		0.981		
		A2	5.660	5.667	0.913	0.914	
			5.680		0.916		
			5.660		0.913		
		A3	9.580	9.607	1.545	1.550	
			9.600		1.548		
			9.640		1.555		
10% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	7	A1	6.300	6.300	1.016	1.016	2-Nov-09
			6.300		1.016		
			6.300		1.016		
		A2	7.640	7.707	1.232	1.243	
			7.760		1.252		
			7.720		1.245		
		A3	8.560	8.593	1.381	1.386	
			8.620		1.390		
			8.600		1.387		
10% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	A1	7.640	7.673	1.232	1.238	3-Nov-09
			7.700		1.242		
			7.680		1.239		
		A2	9.880	9.880	1.594	1.594	

			9.880		1.594		
			9.880		1.594		
			9.200		1.484		
		A3	9.260	9.207	1.494	1.485	
			9.160		1.478		
10% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
			8.960		1.445		
		A1	8.980	8.987	1.448	1.450	
			9.020		1.455		
			11.500		1.855		
	9	A2	11.500	11.507	1.855	1.856	4-Nov-09
			11.520		1.858		
			9.120		1.471		
		A3	9.160	9.147	1.478	1.475	
			9.160		1.478		
10% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
			8.820		1.423		
		A1	8.820	8.820	1.423	1.423	
			8.820		1.423		
			12.540		2.023		
	10	A2	12.540	12.540	2.023	2.023	5-Nov-09
			12.540		2.023		
			10.200		1.645		
		A3	10.200	10.200	1.645	1.645	
			10.200		1.645		

Table I.4: Data for Culture Grown on 15% CO_2.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
			0.260		0.042		
		B1	0.260	0.260	0.042	0.042	
			0.260		0.042		
			0.251		0.040		
15% CO_2	0	B2	0.253	0.253	0.041	0.041	26-Oct-09
			0.255		0.041		
			0.254		0.041		
		B3	0.256	0.255	0.041	0.041	
			0.255		0.041		
15% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	1	B1	0.641	0.640	0.103	0.103	27-Oct-09

			0.640		0.103		
			0.638		0.103		
		B2	0.625	0.625	0.101	0.101	
			0.625		0.101		
			0.625		0.101		
		B3	0.537	0.536	0.087	0.087	
			0.536		0.086		
			0.536		0.086		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
15% CO_2	2	B1	1.002	1.002	0.162	0.162	28-Oct-09
			1.002		0.162		
			1.002		0.162		
		B2	1.210	1.210	0.195	0.195	
			1.210		0.195		
			1.210		0.195		
		B3	1.093	1.093	0.176	0.176	
			1.093		0.176		
			1.093		0.176		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
15% CO_2	3	B1	2.930	2.940	0.473	0.474	29-Oct-09
			2.940		0.474		
			2.950		0.476		
		B2	3.300	3.307	0.532	0.533	
			3.310		0.534		
			3.310		0.534		
		B3	3.680	3.657	0.594	0.590	
			3.640		0.587		
			3.650		0.589		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
15% CO_2	4	B1	3.230	3.250	0.521	0.524	30-Oct-09
			3.250		0.524		
			3.270		0.527		
		B2	4.360	4.367	0.703	0.704	
			4.370		0.705		
			4.370		0.705		
		B3	4.370	4.353	0.705	0.702	
			4.350		0.702		
			4.340		0.700		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
15% CO_2	5	B1	5.520	5.520	0.890	0.890	31-Oct-09

			5.520		0.890		
			5.520		0.890		
		B2	7.540	7.553	1.216	1.218	
			7.560		1.219		
			7.560		1.219		
		B3	9.580	9.607	1.545	1.550	
			9.600		1.548		
			9.640		1.555		
15% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	B1	6.740	6.707	1.087	1.082	1-Nov-09
			6.720		1.084		
			6.660		1.074		
		B2	7.660	7.687	1.236	1.240	
			7.720		1.245		
			7.680		1.239		
		B3	11.120	11.173	1.794	1.802	
			11.220		1.810		
			11.180		1.803		
15% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	7	B1	6.320	6.333	1.019	1.022	2-Nov-09
			6.340		1.023		
			6.340		1.023		
		B2	9.080	9.080	1.465	1.465	
			9.080		1.465		
			9.080		1.465		
		B3	12.840	12.867	2.071	2.075	
			12.860		2.074		
			12.900		2.081		
15% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	B1	7.600	7.587	1.226	1.224	3-Nov-09
			7.560		1.219		
			7.600		1.226		
		B2	11.000	11.033	1.774	1.780	
			11.040		1.781		
			11.060		1.784		
		B3	11.660	11.640	1.881	1.878	
			11.660		1.881		
			11.600		1.871		
15% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	9	B1	9.980	9.987	1.610	1.611	4-Nov-09

			10.000		1.613		
			9.980		1.610		
		B2	12.740		2.055		
			12.720	12.707	2.052	2.050	
			12.660		2.042		
		B3	11.300		1.823		
			11.300	11.293	1.823	1.822	
			11.280		1.819		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		B1	8.420		1.358		
			8.420	8.420	1.358	1.358	
			8.420		1.358		
15% CO_2		B2	12.640		2.039		
	10		12.640	12.640	2.039	2.039	5-Nov-09
			12.640		2.039		
		B3	10.340		1.668		
			10.340	10.340	1.668	1.668	
			10.340		1.668		

Table I.5: Data for Culture Grown on 20% CO_2.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		C1	0.240		0.039		
			0.239	0.240	0.039	0.039	
			0.242		0.039		
		C2	0.250		0.040		
20% CO_2	0		0.250	0.250	0.040	0.040	26-Oct-09
			0.249		0.040		
		C3	0.249		0.040		
			0.249	0.249	0.040	0.040	
			0.249		0.040		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		C1	0.470		0.076		
			0.467	0.468	0.075	0.076	
			0.468		0.075		
		C2	0.429		0.069		
20% CO_2	1		0.432	0.431	0.070	0.069	27-Oct-09
			0.431		0.070		
		C3	0.460		0.074		
			0.455	0.458	0.073	0.074	

			0.459		0.074		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
20% CO_2	2	C1	0.724	0.721	0.117	0.116	28-Oct-09
			0.718		0.116		
			0.722		0.116		
		C2	0.707	0.707	0.114	0.114	
			0.707		0.114		
			0.707		0.114		
		C3	0.757	0.758	0.122	0.122	
			0.758		0.122		
			0.758		0.122		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
20% CO_2	3	C1	1.840	1.840	0.297	0.297	17-Oct-09
			1.840		0.297		
			1.840		0.297		
		C2	1.650	1.640	0.266	0.265	
			1.640		0.265		
			1.630		0.263		
		C3	1.840	1.830	0.297	0.295	
			1.830		0.295		
			1.820		0.294		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
20% CO_2	4	C1	2.970	2.993	0.479	0.483	30-Oct-09
			3.010		0.486		
			3.000		0.484		
		C2	2.760	2.737	0.445	0.441	
			2.720		0.439		
			2.730		0.440		
		C3	2.260	2.287	0.365	0.369	
			2.290		0.369		
			2.310		0.373		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
20% CO_2	5	C1	6.000	6.000	0.968	0.968	31-Oct-09
			5.980		0.965		
			6.020		0.971		
		C2	4.480	4.473	0.723	0.722	
			4.480		0.723		
			4.460		0.719		
		C3	3.260	3.300	0.526	0.532	
			3.300		0.532		

			3.340		0.539		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
20% CO_2	6	C1	7.940	8.000	1.281	1.290	1-Nov-09
			8.020		1.294		
			8.040		1.297		
		C2	6.360	6.347	1.026	1.024	
			6.300		1.016		
			6.380		1.029		
		C3	4.920	4.847	0.794	0.782	
			4.780		0.771		
			4.840		0.781		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
20% CO_2	7	C1	6.920	6.940	1.116	1.119	2-Nov-09
			6.920		1.116		
			6.980		1.126		
		C2	6.520	6.460	1.052	1.042	
			6.420		1.036		
			6.440		1.039		
		C3	5.160	5.127	0.832	0.827	
			5.100		0.823		
			5.120		0.826		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
20% CO_2	8	C1	8.720	8.720	1.407	1.407	3-Nov-09
			8.720		1.407		
			8.720		1.407		
		C2	8.420	8.393	1.358	1.354	
			8.360		1.348		
			8.400		1.355		
		C3	6.260	6.280	1.010	1.013	
			6.280		1.013		
			6.300		1.016		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
20% CO_2	9	C1	10.880	10.853	1.755	1.751	4-Nov-09
			10.840		1.748		
			10.840		1.748		
		C2	10.520	10.520	1.697	1.697	
			10.520		1.697		
			10.520		1.697		
		C3	7.420	7.460	1.197	1.203	
			7.500		1.210		

			7.460		1.203		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
20% CO_2	10	C1	10.620	10.620	1.713	1.713	5-Nov-09
			10.620		1.713		
			10.620		1.713		
		C2	9.300	9.300	1.500	1.500	
			9.300		1.500		
			9.300		1.500		
		C3	7.280	7.280	1.174	1.174	
			7.280		1.174		
			7.280		1.174		

Table I.6: Data for Culture Grown on 25% CO_2.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	0	A1	0.215	0.215	0.035	0.035	9-Nov-09
			0.215		0.035		
			0.215		0.035		
		A2	0.209	0.209	0.034	0.034	
			0.209		0.034		
			0.209		0.034		
		A3	0.213	0.213	0.034	0.034	
			0.213		0.034		
			0.213		0.034		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	1	A1	0.442	0.442	0.071	0.071	10-Nov-09
			0.442		0.071		
			0.441		0.071		
		A2	0.421	0.421	0.068	0.068	
			0.422		0.068		
			0.421		0.068		
		A3	0.433	0.436	0.070	0.070	
			0.438		0.071		
			0.436		0.070		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	2	A1	0.685	0.687	0.110	0.111	11-Nov-09
			0.688		0.111		
			0.687		0.111		
		A2	0.721	0.720	0.116	0.116	
			0.719		0.116		

			0.719		0.116		
		A3	0.768	0.768	0.124	0.124	
			0.768		0.124		
			0.767		0.124		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	3	A1	1.655	1.657	0.267	0.267	12-Nov-09
			1.645		0.265		
			1.670		0.269		
		A2	1.700	1.705	0.274	0.275	
			1.705		0.275		
			1.710		0.276		
		A3	1.800	1.766	0.290	0.285	
			1.815		0.293		
			1.682		0.271		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	4	A1	2.350	2.343	0.379	0.378	13-Nov-09
			2.345		0.378		
			2.335		0.377		
		A2	2.485	2.405	0.401	0.388	
			2.249		0.363		
			2.480		0.400		
		A3	2.725	2.725	0.440	0.440	
			2.725		0.440		
			2.725		0.440		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	5	A1	3.270	3.250	0.527	0.524	14-Nov-09
			3.250		0.524		
			3.230		0.521		
		A2	3.660	3.673	0.590	0.593	
			3.680		0.594		
			3.680		0.594		
		A3	3.900	3.917	0.629	0.632	
			3.910		0.631		
			3.940		0.636		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	6	A1	3.720	3.723	0.600	0.601	15-Nov-09
			3.730		0.602		
			3.720		0.600		
		A2	5.310	5.313	0.857	0.857	
			5.330		0.860		

			5.300		0.855		
		A3	4.760	4.770	0.768	0.769	
			4.770		0.769		
			4.780		0.771		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	7	A1	3.670	3.667	0.592	0.591	16-Nov-09
			3.660		0.590		
			3.670		0.592		
		A2	6.420	6.427	1.036	1.037	
			6.420		1.036		
			6.440		1.039		
		A3	5.680	5.667	0.916	0.914	
			5.650		0.911		
			5.670		0.915		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	8	A1	5.220	5.247	0.842	0.846	17-Nov-09
			5.240		0.845		
			5.280		0.852		
		A2	6.240	6.267	1.007	1.011	
			6.300		1.016		
			6.260		1.010		
		A3	5.580	5.613	0.900	0.905	
			5.620		0.907		
			5.640		0.910		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	9	A1	6.740	6.740	1.087	1.087	18-Nov-09
			6.720		1.084		
			6.760		1.090		
		A2	6.760	6.753	1.090	1.089	
			6.760		1.090		
			6.740		1.087		
		A3	6.900	6.893	1.113	1.112	
			6.920		1.116		
			6.860		1.107		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
25% CO_2	10	A1	8.920	8.860	1.439	1.429	19-Nov-09
			8.880		1.432		
			8.780		1.416		
		A2	6.920	6.927	1.116	1.117	
			6.940		1.119		

			6.920		1.116		
		A3	7.140	7.113	1.152	1.147	
			7.120		1.148		
			7.080		1.142		

Table I.7: Data for Culture Grown on 30% CO_2.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
30% CO_2	0	B1	0.210	0.210	0.034	0.034	9-Nov-09
			0.210		0.034		
			0.210		0.034		
		B2	0.219	0.219	0.035	0.035	
			0.219		0.035		
			0.219		0.035		
		B3	0.220	0.220	0.035	0.035	
			0.220		0.035		
			0.220		0.035		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
30% CO_2	1	B1	0.359	0.359	0.058	0.058	10-Nov-09
			0.359		0.058		
			0.359		0.058		
		B2	0.364	0.363	0.059	0.059	
			0.362		0.058		
			0.363		0.059		
		B3	0.431	0.433	0.070	0.070	
			0.433		0.070		
			0.434		0.070		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
30% CO_2	2	B1	0.528	0.527	0.085	0.085	11-Nov-09
			0.526		0.085		
			0.527		0.085		
		B2	0.482	0.482	0.078	0.078	
			0.483		0.078		
			0.482		0.078		
		B3	0.548	0.548	0.088	0.088	
			0.548		0.088		
			0.548		0.088		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
30% CO_2	3	B1	0.860	0.863	0.139	0.139	12-Nov-09
			0.865		0.140		

			0.865		0.140		
		B2	0.700	0.705	0.113	0.114	
			0.705		0.114		
			0.710		0.115		
		B3	1.545	1.548	0.249	0.250	
			1.550		0.250		
			1.550		0.250		
30% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	B1	1.310	1.303	0.211	0.210	13-Nov-09
			1.305		0.210		
			1.295		0.209		
		B2	0.910	0.917	0.147	0.148	
			0.915		0.148		
			0.925		0.149		
		B3	1.545	1.548	0.249	0.250	
			1.550		0.250		
			1.550		0.250		
30% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	5	B1	2.060	2.037	0.332	0.329	14-Nov-09
			2.030		0.327		
			2.020		0.326		
		B2	2.100	2.127	0.339	0.343	
			2.130		0.344		
			2.150		0.347		
		B3	3.500	3.533	0.565	0.570	
			3.560		0.574		
			3.540		0.571		
30% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	B1	2.660	2.670	0.429	0.431	15-Nov-09
			2.660		0.429		
			2.690		0.434		
		B2	3.460	3.447	0.558	0.556	
			3.430		0.553		
			3.450		0.556		
		B3	4.400	4.413	0.710	0.712	
			4.410		0.711		
			4.430		0.715		
30% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	7	B1	2.630	2.613	0.424	0.422	16-Nov-09
			2.610		0.421		

			2.600		0.419		
		B2	3.700	3.717	0.597	0.599	
			3.720		0.600		
			3.730		0.602		
		B3	5.610	5.623	0.905	0.907	
			5.620		0.907		
			5.640		0.910		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
30% CO_2	8	B1	3.460	3.473	0.558	0.560	17-Nov-09
			3.480		0.561		
			3.480		0.561		
		B2	3.980	4.020	0.642	0.648	
			4.020		0.648		
			4.060		0.655		
		B3	7.660	7.667	1.236	1.237	
			7.680		1.239		
			7.660		1.236		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
30% CO_2	9	B1	5.140	5.140	0.829	0.829	18-Nov-09
			5.120		0.826		
			5.160		0.832		
		B2	4.360	4.353	0.703	0.702	
			4.340		0.700		
			4.360		0.703		
		B3	8.600	8.580	1.387	1.384	
			8.580		1.384		
			8.560		1.381		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
30% CO_2	10	B1	5.300	5.307	0.855	0.856	19-Nov-09
			5.300		0.855		
			5.320		0.858		
		B2	4.020	4.040	0.648	0.652	
			4.040		0.652		
			4.060		0.655		
		B3	9.320	9.293	1.503	1.499	
			9.260		1.494		
			9.300		1.500		

Table I.8: Data for Culture Grown on 35% CO_2.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	0	C1	0.207	0.207	0.033	0.033	9-Nov-09
			0.207		0.033		
			0.207		0.033		
		C2	0.206	0.206	0.033	0.033	
			0.206		0.033		
			0.206		0.033		
		C3	0.207	0.207	0.033	0.033	
			0.207		0.033		
			0.207		0.033		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	1	C1	0.387	0.387	0.062	0.062	10-Nov-09
			0.387		0.062		
			0.388		0.063		
		C2	0.354	0.354	0.057	0.057	
			0.353		0.057		
			0.354		0.057		
		C3	0.371	0.373	0.060	0.060	
			0.374		0.060		
			0.375		0.060		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	2	C1	0.553	0.552	0.089	0.089	11-Nov-09
			0.552		0.089		
			0.552		0.089		
		C2	0.520	0.521	0.084	0.084	
			0.521		0.084		
			0.522		0.084		
		C3	0.602	0.603	0.097	0.097	
			0.603		0.097		
			0.604		0.097		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	3	C1	1.065	1.073	0.172	0.173	12-Nov-09
			1.075		0.173		
			1.080		0.174		
		C2	1.035	1.028	0.167	0.166	
			1.020		0.165		
			1.030		0.166		
		C3	1.135	1.128	0.183	0.182	

			1.115		0.180		
			1.135		0.183		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	4	C1	1.935	1.930	0.312	0.311	13-Nov-09
			1.930		0.311		
			1.925		0.311		
		C2	1.580	1.583	0.255	0.255	
			1.580		0.255		
			1.590		0.256		
		C3	1.690	1.685	0.273	0.272	
			1.685		0.272		
			1.680		0.271		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	5	C1	3.400	3.413	0.548	0.551	14-Nov-09
			3.430		0.553		
			3.410		0.550		
		C2	2.630	2.633	0.424	0.425	
			2.630		0.424		
			2.640		0.426		
		C3	2.440	2.427	0.394	0.391	
			2.430		0.392		
			2.410		0.389		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	6	C1	4.200	4.200	0.677	0.677	15-Nov-09
			4.200		0.677		
			4.200		0.677		
		C2	3.180	3.197	0.513	0.516	
			3.200		0.516		
			3.210		0.518		
		C3	2.820	2.807	0.455	0.453	
			2.810		0.453		
			2.790		0.450		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	7	C1	4.720	4.730	0.761	0.763	16-Nov-09
			4.730		0.763		
			4.740		0.765		
		C2	3.550	3.550	0.573	0.573	
			3.540		0.571		
			3.560		0.574		
		C3	2.720	2.720	0.439	0.439	

			2.720		0.439		
			2.720		0.439		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	8	C1	7.040	7.080	1.136	1.142	17-Nov-09
			7.080		1.142		
			7.120		1.148		
		C2	4.500	4.480	0.726	0.723	
			4.480		0.723		
			4.460		0.719		
		C3	3.840	3.860	0.619	0.623	
			3.860		0.623		
			3.880		0.626		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	9	C1	7.500	7.500	1.210	1.210	18-Nov-09
			7.520		1.213		
			7.480		1.207		
		C2	5.220	5.220	0.842	0.842	
			5.220		0.842		
			5.220		0.842		
		C3	4.020	4.060	0.648	0.655	
			4.060		0.655		
			4.100		0.661		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
35% CO_2	10	C1	7.600	7.607	1.226	1.227	19-Nov-09
			7.600		1.226		
			7.620		1.229		
		C2	5.440	5.407	0.877	0.872	
			5.400		0.871		
			5.380		0.868		
		C3	3.780	3.753	0.610	0.605	
			3.760		0.606		
			3.720		0.600		

Table I.9: Data for Culture Grown on 50% CO_2.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
50% CO_2	0	C1	0.269	0.269	0.043	0.043	14-Oct-09
			0.269		0.043		
			0.269		0.043		
		C2	0.268	0.269	0.043	0.043	

			0.269		0.043		
			0.270		0.044		
		C3	0.270		0.044		
			0.269	0.269	0.043	0.043	
			0.269		0.043		
50% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	1	C1	0.329		0.053		
			0.332	0.331	0.054	0.053	
			0.331		0.053		
		C2	0.339		0.055		
			0.339	0.339	0.055	0.055	15-Oct-09
			0.339		0.055		
		C3	0.338		0.055		
			0.338	0.338	0.055	0.055	
			0.338		0.055		
50% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	2	C1	0.441		0.071		
			0.441	0.441	0.071	0.071	
			0.441		0.071		
		C2	0.363		0.059		
			0.361	0.362	0.058	0.058	16-Oct-09
			0.361		0.058		
		C3	0.421		0.068		
			0.421	0.421	0.068	0.068	
			0.422		0.068		
50% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	3	C1	0.638		0.103		
			0.644	0.641	0.104	0.103	
			0.642		0.104		
		C2	0.484		0.078		
			0.482	0.483	0.078	0.078	17-Oct-09
			0.484		0.078		
		C3	0.644		0.104		
			0.638	0.640	0.103	0.103	
			0.638		0.103		
50% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	4	C1	0.710		0.115		
			0.704	0.707	0.114	0.114	
			0.706		0.114		18-Oct-09
		C2	0.534	0.536	0.086	0.086	

			0.536		0.086		
			0.538		0.087		
		C3	0.838		0.135		
			0.850	0.845	0.137	0.136	
			0.848		0.137		
50% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	5	C1	0.769		0.124		
			0.765	0.767	0.123	0.124	
			0.766		0.124		
		C2	0.592		0.095		
			0.589	0.590	0.095	0.095	19-Oct-09
			0.588		0.095		
		C3	1.022		0.165		
			1.021	1.023	0.165	0.165	
			1.026		0.165		
50% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	6	C1	0.707		0.114		
			0.710	0.709	0.115	0.114	
			0.711		0.115		
		C2	0.598		0.096		
			0.601	0.600	0.097	0.097	20-Oct-09
			0.602		0.097		
		C3	0.601		0.097		
			0.607	0.604	0.098	0.097	
			0.603		0.097		
50% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	7	C1	0.942		0.152		
			0.946	0.944	0.153	0.152	
			0.945		0.152		
		C2	0.674		0.109		
			0.679	0.676	0.110	0.109	21-Oct-09
			0.675		0.109		
		C3	0.808		0.130		
			0.806	0.808	0.130	0.130	
			0.809		0.130		
50% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	8	C1	1.288		0.208		
			1.294	1.291	0.209	0.208	22-Oct-09
			1.291		0.208		
		C2	0.777	0.775	0.125	0.125	

			0.774		0.125		
			0.775		0.125		
		C3	0.667		0.108		
			0.673	0.670	0.109	0.108	
			0.669		0.108		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		C1	2.500		0.403		
			2.496	2.499	0.403	0.403	
			2.500		0.403		
50% CO_2		C2	1.164		0.188		
	9		1.164	1.164	0.188	0.188	23-Oct-09
			1.164		0.188		
		C3	1.384		0.223		
			1.388	1.389	0.224	0.224	
			1.396		0.225		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		C1	3.450		0.556		
			3.415	3.430	0.551	0.553	
			3.425		0.552		
50% CO_2		C2	0.835		0.135		
	10		0.865	0.858	0.140	0.138	24-Oct-09
			0.875		0.141		
		C3	2.890		0.466		
			2.865	2.887	0.462	0.466	
			2.905		0.469		

Table I.10: Data for Culture Grown on 100% CO_2.

	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
		D1	0.257		0.041		
			0.257	0.257	0.041	0.042	
			0.258		0.042		
100% CO_2		D2	0.273		0.044		
	0		0.273	0.273	0.044	0.044	14-Oct-09
			0.273		0.044		
		D3	0.263		0.042		
			0.264	0.264	0.043	0.043	
			0.265		0.043		
100% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date

		D1	0.232	0.232	0.037	0.037	
			0.232		0.037		
			0.232		0.037		
	1	D2	0.225	0.225	0.036	0.036	15-Oct-09
			0.225		0.036		
			0.224		0.036		
		D3	0.222	0.221	0.036	0.036	
			0.220		0.035		
			0.221		0.036		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
100% CO_2		D1	0.201	0.202	0.032	0.033	
			0.203		0.033		
			0.203		0.033		
	2	D2	0.201	0.201	0.032	0.032	16-Oct-09
			0.203		0.033		
			0.200		0.032		
		D3	0.216	0.216	0.035	0.035	
			0.216		0.035		
			0.216		0.035		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
100% CO_2		D1	0.205	0.209	0.033	0.034	
			0.211		0.034		
			0.210		0.034		
	3	D2	0.217	0.219	0.035	0.035	17-Oct-09
			0.220		0.035		
			0.220		0.035		
		D3	0.269	0.268	0.043	0.043	
			0.268		0.043		
			0.268		0.043		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
100% CO_2		D1	0.202	0.201	0.033	0.032	
			0.201		0.032		
			0.201		0.032		
	4	D2	0.187	0.189	0.030	0.031	18-Oct-09
			0.190		0.031		
			0.191		0.031		
		D3	0.287	0.286	0.046	0.046	
			0.285		0.046		
			0.286		0.046		
100% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date

			0.219		0.035		
		D1	0.216	0.217	0.035	0.035	
			0.215		0.035		
			0.178		0.029		
	5	D2	0.181	0.179	0.029	0.029	19-Oct-09
			0.179		0.029		
			0.293		0.047		
		D3	0.296	0.295	0.048	0.048	
			0.297		0.048		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
			0.198		0.032		
		D1	0.196	0.197	0.032	0.032	
			0.196		0.032		
			0.174		0.028		
100% CO_2	6	D2	0.174	0.174	0.028	0.028	20-Oct-09
			0.174		0.028		
			0.188		0.030		
		D3	0.189	0.189	0.030	0.030	
			0.189		0.030		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
			0.212		0.034		
		D1	0.211	0.211	0.034	0.034	
			0.211		0.034		
			0.183		0.030		
100% CO_2	7	D2	0.182	0.182	0.029	0.029	21-Oct-09
			0.182		0.029		
			0.249		0.040		
		D3	0.249	0.249	0.040	0.040	
			0.249		0.040		
	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
			0.232		0.037		
		D1	0.237	0.235	0.038	0.038	
			0.236		0.038		
			0.176		0.028		
100% CO_2	8	D2	0.176	0.176	0.028	0.028	22-Oct-09
			0.177		0.029		
			0.177		0.029		
		D3	0.182	0.181	0.029	0.029	
			0.184		0.030		
100% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date

	9	D1	0.266	0.259	0.043	0.042	23-Oct-09
			0.254		0.041		
			0.258		0.042		
		D2	0.192	0.189	0.031	0.030	
			0.188		0.030		
			0.187		0.030		
		D3	0.250	0.251	0.040	0.040	
			0.251		0.040		
			0.251		0.040		
100% CO_2	Day	Culture Vessel	A550	A550 Mean	Cell Conc. (g/L)	Cell Conc. Mean	Date
	10	D1	0.245	0.246	0.040	0.040	24-Oct-09
			0.247		0.040		
			0.246		0.040		
		D2	0.190	0.188	0.031	0.030	
			0.188		0.030		
			0.187		0.030		
		D3	0.216	0.214	0.035	0.035	
			0.214		0.035		
			0.213		0.034		

Appendix J: Nutrient Data

Table J.1: Bold's Basal Medium (BBM) Recipe and Nutrient Composition.

Component	Stock Solution (mL)	Quantity (mL)	Molar Conc. In Final Medium (M)
Macronutrients			
$NaNO_3$	100.00	10.00	1.17E-02
$CaCl_2 * 2 H_2O$	2.50	10.00	1.70E-04
$MgSO_4 * 7 H_2O$	7.50	10.00	3.04E-04
K_2HPO_4	7.50	10.00	4.31E-04
KH_2PO_4	17.50	10.00	1.29E-03
NaCl	2.50	10.00	4.28E-04
Alkaline EDTA Solution		1.00	
EDTA Anhydrous	50.00		1.71E-04
KOH	31.00		5.53E-04
Acidified Iron Solution		1.00	
$FeSO_4 * 7 H_2O$	4.98		4.48E-05
H_2SO_4 (Conc.)	1 mL		
Boron Solution		1.00	
H_3BO_3	11.42		4.62E-04
Trace Metal Solution		1.00	
$ZnSO_4 * 7 H_2O$	8.82		7.67E-05
$MnCl_2 * 4 H_2O$	1.44		1.82E-05
MoO_3	0.71		1.23E-05
$CuSO_4 * 5 H_2O$	1.57		1.57E-05
$Co(NO_3)_2 * 6 H_2O$	0.49		4.21E-06

Table J.2: *Abridged* City of Dayton Tap Water Profile.

2008 Water Quality Averages & Pumping Data Summary Full report located @ http://water.cityofdayton.org/Water/docs/2008summaryPart1.pdf	
CHEMICAL ANALYSIS	**DISTRIBUTION SYSTEM (mg/L)**
Total Hardness as CaCO3	**152.5**
P. Alk. as CaCO3	**5.0**
Total Alk. as CaCO3	**84.5**
Non-Carb. Hard. as CaCO3	**68.0**

Ca. Hard. as CaCO3	**66.8**
Mg. Hard. as CaCO3	**85.8**
Calcium	**26.7**
Magnesium	**20.8**
Sulfate	**50.4**
Chloride	**53.9**
Nitrate & Nitrite	**1.0**
Nitrite	**<0.05**
Sodium	**26.6**
Potassium	**2.9**
Chlorine - Free	**1.125**
Chlorine - Total	**1.213**
Total Organic Carbon	**0.65**
MICROBIOLOGICAL	
Total Coliform, % Positive	**0.00**
E. coli, % Positive	**0.00**
HPC colonies/100ml	**29.09**
Cryptosporidum & Giardia	**None**

Table J.3: Scott's Peters® Excel® Cal-Mag 15-5-15 Nutrient Concentration and Amounts (used in Experiment 2).

Nutrient	Percentage (%)	MW of Nutrient in Question (g/mol)	Solution containing 1 g/L Cal-Mag (mg/L)	Concentration of Nutrient in 1 g/L Cal-Mag (mmol/L)	Solution containing 2 g/L Cal-Mag (mg/L)	Concentration of Nutrient 2 g/L Cal-Mag (mmol/L)	Solution containing 5 g/L Cal-Mag (mg/L)	Concentration of Nutrient 5g/L Cal-Mag (mmol/L)
NH_3 - N	1.200	14.00	12	**0.857**	24	**1.714**	60	**4.286**
NO_3 - N	11.750	14.00	117.5	**8.393**	235	**16.786**	587.5	**41.964**
Urea - N	2.050	14.00	20.5	**2.929**	41	**5.857**	102.5	**14.643**
P_2O_5 - P	5.000	30.97	50	**3.229**	100	**6.458**	250	**16.145**
K_2O - K	15.000	39.098	150	**7.673**	300	**78.196**	750	**19.183**
CaO - Ca	7.000	40.08	70	**1.747**	140	**3.493**	350	**8.733**
MgO - Mg	3.000	24.305	30	**1.234**	60	**2.469**	150	**6.172**
Boron	0.015	10.81	0.15	**0.014**	0.3	**0.028**	0.75	**0.069**
Copper	0.007	63.55	0.07	**0.001**	0.14	**0.002**	0.35	**0.006**
Iron	0.075	55.85	0.75	**0.013**	1.5	**0.027**	3.75	**0.067**
Manganese	0.037	54.94	0.37	**0.007**	0.74	**0.013**	1.85	**0.034**
Molybdenum	0.007	95.94	0.07	**0.001**	0.14	**0.001**	0.35	**0.004**
Zinc	0.040	65.39	0.4	**0.006**	0.8	**0.012**	2	**0.031**

Table J.4: Commercial Fertilizer Nutrient Concentrations and Amounts (prepared according to Bold's Recipe and used in Experiments 4 and 5).

Nutrient	Concentration Needed (mmol/L) to equal BBM4N	Comm. Fert. Nutrient Source	MW (g/mol)	Amount of Nutrient Required (mg/L) for Medium Solution		
NO_3 - N	0.01176	KNO_3	101.1	**1188.936**		
PO_4 - P	0.00129	KH_2PO_4	136.09	**175.5561**		
PO_4 - P	0.000431	$K_2HPO_4 * 3H_2O$	228.22	**98.36282**		
Ca	0.00017	$Ca(NO_3)_2 * 4H_2O$	236.1	**40.137**		
Mg	0.000304	$MgSO_4 * 7H_2O$	120.37	**36.59248**		
EDTA	0.000171	EDTA	292.24	**49.97304**		
		STEM^		**0.75 g/L STEM**	**% of STEM Nutrient Represents**	**% of BBM**
Fe	2.024E-01	$Fe(II)SO_4 * 7H_2O$	277.91	**0.0563**	7.5	451.7857143
B	1.638E-01	H_3BO_3	61.83	**0.0101**	1.35	35.45454545
Zn	1.174E-01	$ZnSO_4 * 7H_2O$	287.45	**0.0338**	4.5	153.06
Mn	3.974E-01	$MnSO_4$	151	**0.0600**	8	2183.516484
Mo	1.240E-03	$Na_2MoO_4 * 2H_2O$	241.92	**0.0003**	0.04	10.08
Cu	6.911E-02	$CuSO_4 * 5H_2O$	249.61	**0.0173**	2.3	440.1910828

Ingredients are added to 1 L of water

^Soluble Trace Element Mixture

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14. ABSTRACT

The micro-alga *Chlorella vulgaris* was cultivated under a variety of environmental conditions in various culture media solutions to optimize growth rate and biomass productivity. Efforts during this work investigated growth at the micro-scale in an air-lift bubble system with the goal of interpreting performance characteristics for application to a larger tubular Photo-bioreactor. Maximum growth rates and biomass yields were 0.65 d^{-1} and 2.003 g biomass/L and achieved in seven days using urea in de-ionized water under a 24:0 Photoperiod (Light:Dark). Additionally, growth rates and biomass yields of 0.65 d^{-1} and 1.964 g biomass/L were achieved over the same time period using commercial fertilizers in Charcoal Filtered Tap Water, indicating that the alga is robust and tolerant of a wide range of environmental conditions, including nutrient composition and water type.

CO_2 tolerance was investigated to determine the utility of the alga in power plant flue gas remediation schemes. The alga grew in all CO_2-in-Air concentrations between ambient air and 50% CO_2 with maximum growth occurring at concentrations between ambient levels and 20% CO_2-in-Air. Greatest growth was observed in the culture using 15% CO_2-in-Air, indicating this particular alga may be appropriate for power plant flue gas remediation (13-16% CO_2 in flue gas).

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